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PROCEEDINGS: BIOSTIMULATION/AND/NUTRIENT ASSESSMENT WORKSHOP HELD AT CORVALLIS, OREGON ON 16-17 OCTOBER 1973

Pacific Northwest Environmental Research Laboratory
Corvallis, Oregon

June 1975

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PROCEEDINGS: BIOSTIMULATION - NUTRIENT ASSESSMENT WORKSHOP

October 16-17, 1973
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This report was submitted by the Pacific Northwest Environmental Research Laboratory of the Environmental Protection Agency. The conference was held October 16-17, 1973.

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RESEARCH PERTAINING TO DETERMINATION OF ATP IN SOILS AND SUBSURFACE FORMATIONS

William R Duffer, William J. Dunlap
and James McNabb

The National Water Quality Control Research Program and the National Ground Water Research Program at the Robert S. Kerr Environmental Research Laboratory are concerned with the determination of ATP as a measure of bioactivity in soils and subsurface formations. Such measurements are needed in investigations pertaining to land application of waste waters and degradation of ground water.

Lee et al have developed a method for ATP determination that is moderately successful for sediments and surface soils.(1) This method involved acid extraction of soil or sediment samples, treatment of the extract with cation exchange resin, and determination of ATP in the extracts by the luciferin-luciferase bioluminescence assay. The effect of substances in the extract which interfere with the ATP-luciferin-luciferase reaction is reduced or eliminated by dilution. However, our preliminary investigations indicate that, because of the presence of high levels of interfering substances in extracts of many terrestrial materials, the Lee method lacks the sensitivity required for meaningful analysis of low activity samples, such as those originating in subsurface regions or surface regions receiving noxious wastes.

Present research efforts have been directed toward improvement of the sensitivity of the luciferin-luciferase bioluminescence assay for ATP in terrestrial materials. We have attempted to develop relatively simple and rapid column chromatographic procedures for purification of ATP in acid extracts of soil materials. Acid solutions of ATP uniformly labeled with ^{14}C were chromatographed on columns containing various chromatographic stationary phases. ATP in eluate fractions was detected and quantitated by liquid scintillation spectrometry. Cation exchange, molecular exclusion chromatography, and ion retardation were employed in efforts to retain interfering substances on the column while rapidly eluting ATP. These efforts were unsuccessful because of failure to achieve satisfactory separation of ATP from impurities on reasonably sized columns or because of unacceptable loss of ATP due to sorption on the columns. Chromatographic procedures employing strongly and weakly basic anion exchange resins, neutral polymeric adsorbents, and ion retardation resin were utilized in efforts to rapidly elute interfering substances while retaining ATP on the columns, with subsequent elution of purified ATP. Procedures employing anion exchange resins and neutral polymeric adsorbents were generally unsuccessful due to very strong sorption of ATP on the columns. Such sorption resulted in the need for unacceptably lengthy or drastic procedures for ATP elution. ATP was found to be effectively retained on Bio Rad AG 11 A8 ion retardation resin when applied to the column in 0.6 N H_2SO_4 solution and during subsequent water elution, and appears to be relatively easily eluted from such columns by 0.2 M to 1 M NH_4Cl and Tris Hydrochloride*. Experiments in our laboratories indicate that NH_4Cl and Tris HCl in concentrations required for ATP elution from the ion retardation resin interfere to some extent with the luciferin-luciferase reaction. Nevertheless, this procedure appears to offer some promise for improving the sensitivity of ATP analysis in terrestrial material, and is being further investigated.

Doxtader has developed a butanol extraction method for ATP in soils.(2) This may give a cleaner extract which may be more easily purified for greater sensitivity. We are awaiting details of this method and will examine it experimentally if our present avenue of approach is blocked or if it appears more likely to provide the simplicity and sensitivity desired.

REFERENCES

1. Lee, C. C., R. F. Harris, J. D. H. Williams, D. E. Armstrong, and J. K. Syers. Adenosine Triphosphate in Lake Sediments: I. Determination. Soil Sci. Soc. Amer. Proc. 35:82-86. 1971.
2. Ausmus, B. S. The Use of the ATP Assay in Terrestrial Decomposition Studies. Bull. Ecol. Res. Comm. (Stockholm) 17:223-234. 1973.

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*Tris (hydroxymethyl) aminomethane hydrochloride

THE EFFECT OF MASS TRANSPORT ON BIOSTIMULATION OF ALGAL GROWTH

James W. Falco, Pat C. Kerr, Michael B. Barron
and Donald L. Brockway

INTRODUCTION

The role of mass transport in regulating nutrient availability and the concomitant effect on biological activity has received little emphasis in ecological studies even though this process could be the over-all rate limiting step in many natural ecosystems. Data indicating the importance of mass transport dates to work reported by James[1] in 1928. The growth rate of aquatic plants was stimulated by the increased availability of carbon resulting from either an increased flow rate of the medium or an increased rate of molecular diffusion. Myers'[2] data indicate the presence of resistance to diffusion of CO_2 between the atmosphere and the cell wall of the green alga, Chlorella pyrenoidosa. Similar results were reported by Kerr et al.[3] for the blue-green alga, Anacystis nidulans. This diffusion resistance in turn regulated the growth of both algae.

Diffusion resistance to oxygen and/or organic substrates has been reported for yeast[4] and bacterial films[5,6,7,8,9]. Jernelov and Lann[10] reported that shaking increased the rate of biological methylation of mercury in both eutrophic and oligotrophic lake sediments. Thus diffusion resistance and turbulence are important in regulating the transport of any material to or from the cell.

Biological uptake is generally mediated, or catalyzed, by surface enzymes, which have different kinetics for the uptake of different materials. Organisms vary widely in size and shape and thus in surface to volume ratio. It should be possible to apply to such biological systems certain of the quantitative theories governing the effectiveness of catalyst mass. These mathematical expressions may then be incorporated into ecosystem (or organism) models.

In this paper, a model of the uptake of major nutrients limited by a diffusional resistance around an algal cell is proposed. A series of experiments to demonstrate these limitations is discussed.

Finally, a model for internal resistance that may limit uptake by colonial algae (and aggregated algal cells) is also developed. Also treated is the case of a single limiting nutrient diffusing independently of other material gradients with a non-linear reaction uptake step at the cell wall boundary.

The mathematical formulations used are analogous to those used by Toor[11] and Hudson[12] in solving transient concentration profiles caused by step changes in boundary conditions of coupled reacting and diffusing materials. The formulations differ in geometry from those used by Toor and Hudson.

The formulations used in the aggregate model to describe diffusion into a mass of cells are similar to those developed by Thiele[13] to describe the diffusion into and reaction occurring inside catalyst grains.

The solutions developed are not restricted by the requirement of commutativity of diffusivity and rate coefficient matrices as are previous solutions by Wei[14], but can be applied to any set of coupled constituents.

EXPERIMENTAL

To demonstrate the effect of mass transfer limitations on uptake of major nutrients and growth rate of algae, a series of comparative experiments was undertaken.

Chlorella pyrenoidosa was cultured in pH 5.5 Benson-Fuller nutrient solution containing one mℓ/ℓ of Hunter's trace element solution. The cultures were grown at 22°C under 2000 fc white light (a cool white fluorescent plus incandescent source) in a water-cooled reciprocating shaker. Cultures were gassed with 1, 3, or 5% CO_2 in air (Linde research grade gases) and were shaken at 0, 17, or 33 rpm with an amplitude of 9.4 cm. Capillary tubing or 27-gauge hypodermic needles, or both, were used to meter and equalize the gas flow to cultures.

Aliquots were removed aseptically from each culture at zero time and at 24-hour intervals thereafter. The algae were enumerated by direct microscopic counts at 1500 X using a Petroff-Hauser Bacteria Counter. The cultures were checked for bacterial contamination daily by direct microscopic examination. Any contaminated culture was discarded.

MODEL FOR EXTERNAL MASS-TRANSFER RESISTANCE TO NUTRIENT UPTAKE

In modeling the uptake of nutrients by an algal cell, we separated the cell/nutrient solution system into three zones. First, throughout the bulk of the liquid surrounding the cell, material is turbulently mixed; consequently the nutrient concentrations are uniform in this first zone. Secondly, in a narrow zone adjacent to the cell wall, there is no convective mixing and thus transport of materials is by diffusion only (Figure 1). This region

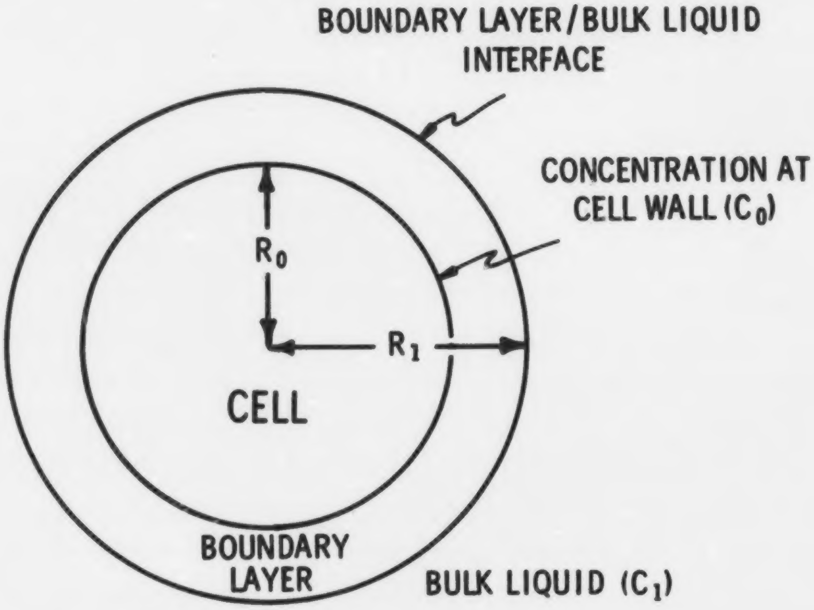
BOUNDARY LAYER/BULK LIQUID INTERFACE
CONCENTRATION AT CELL WALL (C_0)
R_0
R_1
CELL
BOUNDARY LAYER
BULK LIQUID (C_1)

Fig. 1. Model of nutrient diffusion into an algal cell.

is called the boundary layer. Lastly, at the liquid/cell-wall interface, removal of nutrients from the boundary layer proceeds by a kinetic uptake step.

Nutrient transport within the three zones may be described by two boundary conditions and one ordinary differential equation:

$$\frac{1}{r^2}\frac{d}{dr}\left\{r^2\frac{d(C)}{dr}\right\} = 0,\ R_0 \leq r \leq R_1 \tag{1}$$

$$(C) = (C_1),\ r = R_1 \tag{2}$$

$$A\,[D]\,\frac{\partial (C)}{\partial r} = V(R),\ r = R_0 \tag{3}$$

where A = surface area of the cell (μm^2)

(C) = column matrix of nutrient concentrations (g-atom/μm^3)

(C_1) = column matrix of nutrient concentrations in the bulk liquid (g-atom/μm^3)

$[D]$ = matrix of diffusion coefficients (μm^2/sec)

r = distance from the center of an algal cell (μm)

R_1 = distance from the center of the cell to the boundary layer-bulk liquid interface (μm)

R_0 = radius of an algal cell (μm)

(R) = column matrix of specific uptake rates (g-atom/μm^3 sec)

V = volume of an algal cell (μm^3)

Steady-state uptake during a cell cycle has been assumed in order to obtain equation (1), which is a steady-state mass balance. All three equations have been written in matrix form to include the possibility of nutrient uptake coupling between various nutrients either in the diffusion term or in the kinetic uptake term in equation (3).

The only unknown in the three equations is the nutrient uptake matrix in equation (3). If one assumes pseudo-first order uptake during a cell cycle, the rate of uptake would be proportional to the concentration present at the cell wall. The justifications for making such an assumption are (1) the actual non-linear kinetic terms can be linearized to give an apparent first-order rate, and (2) an analytical solution that permits clear discrimination of coupling effects can be obtained. If the uptake rate is assumed to be

$$(R) = [K]\,(C) \qquad (4)$$

where $[K]$ = matrix of rate coefficients (sec^{-1}), the solution to equations (1), (2), and (3) is

$$(C) = \left\{ -\frac{1}{r} \left\{ \frac{3\,[D][K]^{-1}}{R_o^{3}} + \left(\frac{R_1 - R_o}{R_1 R_o}\right)[I] \right\}^{-1} \right.$$

$$\left. + \left\{ [I] + \left\{ \frac{3R_1[D][K]^{-1}}{R_o^{3}} + \left(\frac{R_1 - R_o}{R_o}\right)[I] \right\}^{-1} \right\} (C_1) \right. \qquad (5)$$

where $[I]$ = identity matrix.

As a test case, the boundary layer concentration profiles for *Cyclotella nana*, a diatom for which published data on phosphorus uptake rates are available[15], were calculated. Assuming that the effective radius of an algal cell is 2.65 μm and the boundary layer thickness is approximately 2.65 μm, various profiles result depending on γ, the ratio of D to K, as shown in Figure 2. The scalar form of equation (5) has been assumed, and thus coefficients are the scalar diffusion coefficients of phosphorus in water and the scalar specific uptake rate of phosphorus by *C. nana*. This would occur if phosphorus uptake is limiting and completely uncoupled from the rates of transport and uptake of other materials.

If we assume that phosphorus concentration is below the half saturation constant, K_m, the rate coefficient, K, can be estimated as

$$K = \frac{V_m}{K_m} \qquad (6)$$

where V_m = maximum uptake rate

K_m = half-saturation constant

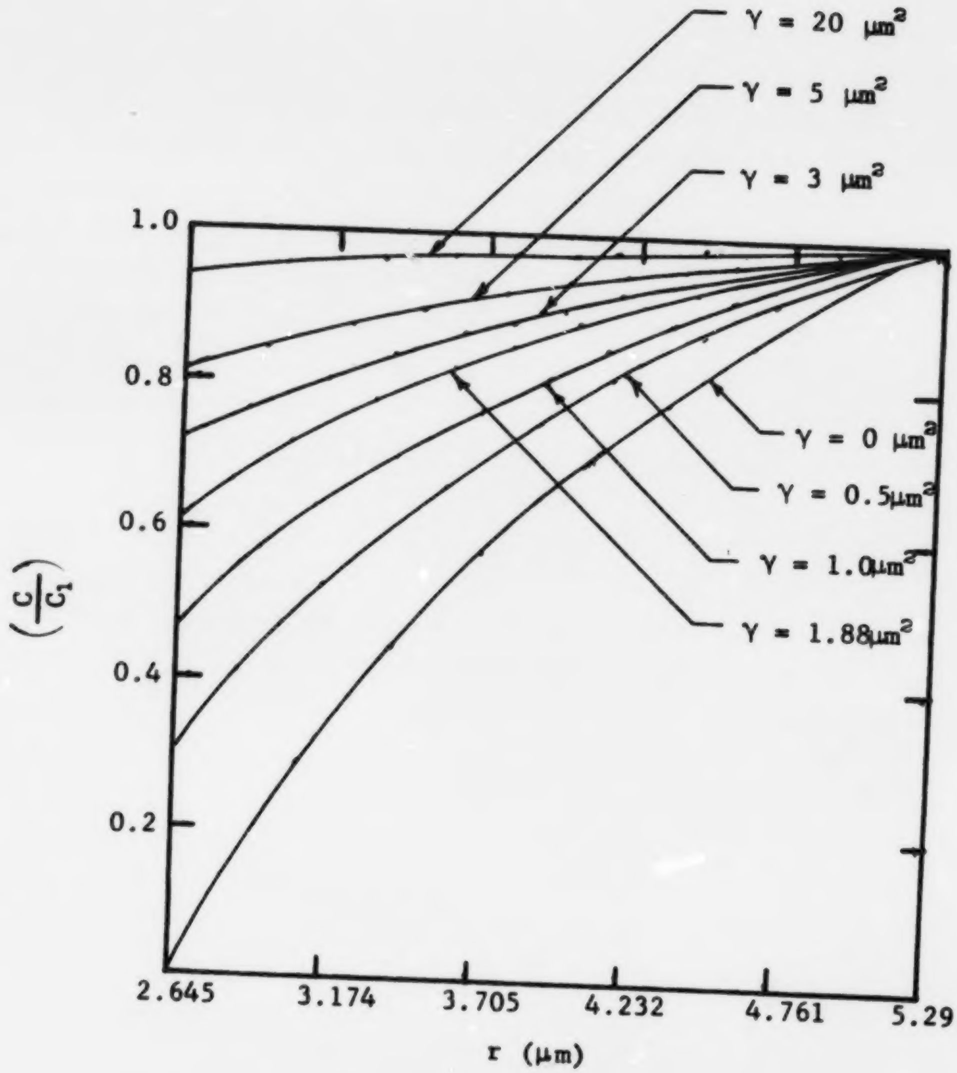

FIGURE 2

BOUNDARY LAYER CONCENTRATION PROFILES AS A FUNCTION OF RADIUS AND RELATIVE UPTAKE RATE (D · K_m/V_m)

For phosphorus uptake by C. nana K is reported by Fuhs[15] to be 53.1 $\sec^{-1}$ where uptake rate has been converted to g-atoms per unit volume of cell per sec. If the diffusion coefficient is assumed to be 100 μm^2/sec (a reasonable estimate), γ is 1.88 μm^2. Referring to Figure 3, this corresponds to an effective reduction in phosphorus uptake of 40% as compared to the case of no diffusional resistance. This is a minimum figure for reduction in uptake rate since it has been assumed that the entire surface area is available for nutrient uptake. In fact, only a portion of the surface occupied by enzyme sites is actually involved. More serious limitations on nutrient transport would therefore exist than is indicated by the above calculations.

If uptake rates at higher concentrations are considered, nonlinear Michaelis-Menten kinetics must be used, i.e.,

$$K = \frac{V_m}{K_m + C} \qquad (7)$$

In this case the form of the solution to equations (1), (2), and (3) is the same as in the linear case but the solution is more complex. Equation (5) becomes

$$C = \left(\frac{1}{R_1} - \frac{1}{r}\right) B + C_1 \qquad (8)$$

where

$$B = \frac{\frac{V_m}{3D}\left(\frac{R_O - R_1}{R_1}\right) - \left(\frac{K_m + C_1}{R_O^2}\right)}{2\,\frac{R_O - R_1}{R_O^3 R_1}}$$

$$+ \frac{\left[\frac{V_m}{3D}\left(\frac{R_O - R_1}{R_1}\right) - \left(\frac{K_m + C_1}{R_O^2}\right)\right]^2 + 4\,\frac{V_m C_i}{3D}\left(\frac{R_O - R_1}{R_O^2 R_1}\right)}{2\,\frac{R_O - R_1}{R_O^3 R_1}} \qquad (9)$$

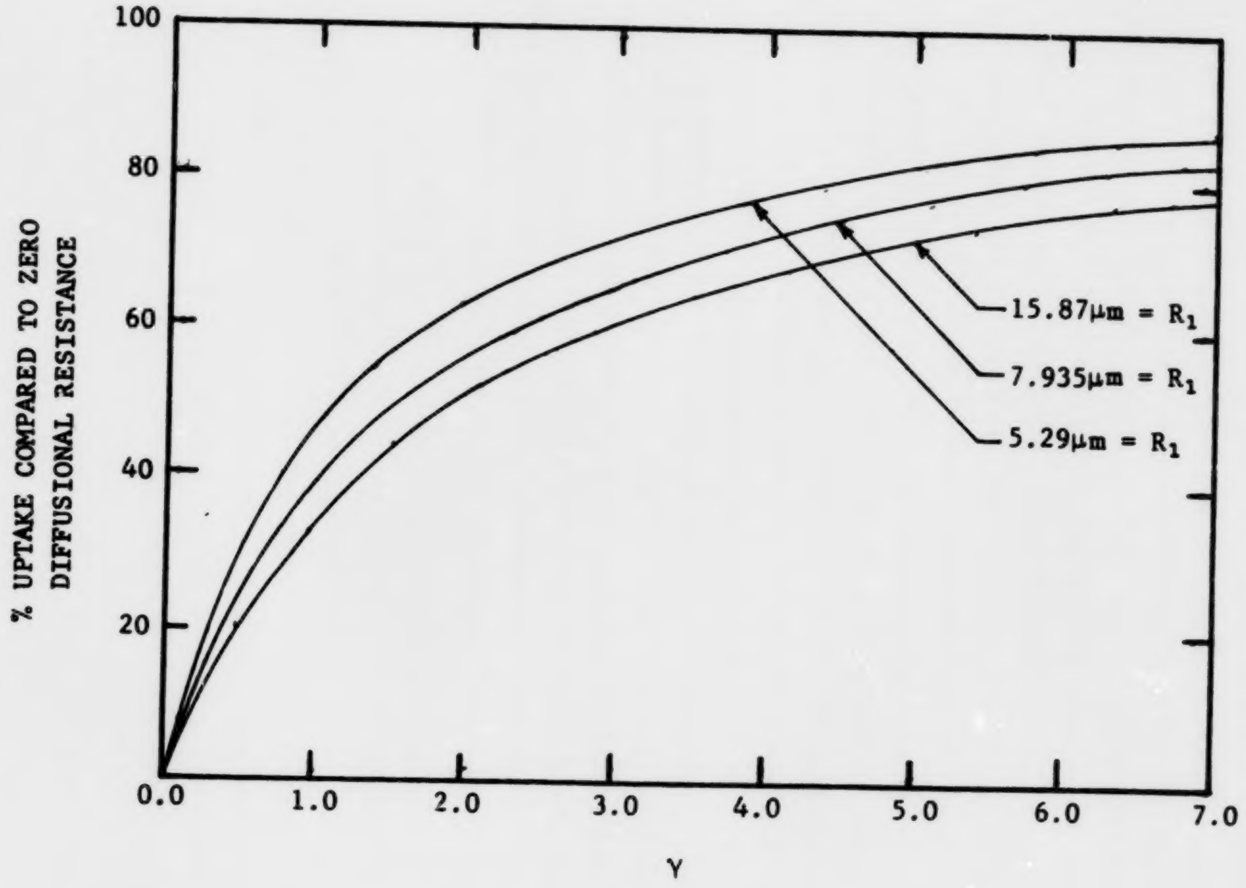

FIGURE 3

REDUCTION IN UPTAKE AS A FUNCTION OF DIFFUSIONAL RESISTANCE

Figure 4 shows a graphical means of estimating B as well as an illustration of nutrient uptake as function of external concentration. The slanted lines represent the relationship between the nutrient concentration in the bulk liquid, nutrient concentration at the cell surface, and the resulting rate of diffusion. To find the cell surface concentration for a given bulk concentration and diffusion rate, find the slanted line whose X intercept corresponds to the bulk concentration. Then follow the slanted line upward to the point at which the Y coordinate equals the diffusion rate. A perpendicular dropped from that point to the X axis indicates the cell surface concentration.

The curved line represents the rate of uptake by the cell as a function of concentration at the cell wall. As stated by equation (3), at steady state the rate of uptake must equal the rate of diffusion or transport to the cell wall. The superimposed graphs of diffusion and uptake therefore represent a graphical solution to the equation. If, for example, the bulk phosphorus concentration is 2.3×10^{-21} g-atom/μm^3, the point at which the rate of diffusion equals the rate of uptake may be found by following the appropriate slanted line to its intersection with the curved uptake line. The X coordinate of the point is the resulting cell wall concentration (C_0) (2.02×10^{-21}) and the Y coordinate is the resulting rate of diffusion and uptake (0.78×10^{-14} g-atom/hr cell), which is equal to B/R_0^2.

Since the rate of uptake approaches a limiting value (V_m) as cell wall concentration increases and the diffusion rate is linearly proportional to the concentration difference (C_1-C_0), diffusion limitations become less important as nutrient concentration increases. Figure 5, showing the ratio of cell wall concentrations (C_0) to bulk concentrations (C_1) as a function of bulk concentration, illustrates this point.

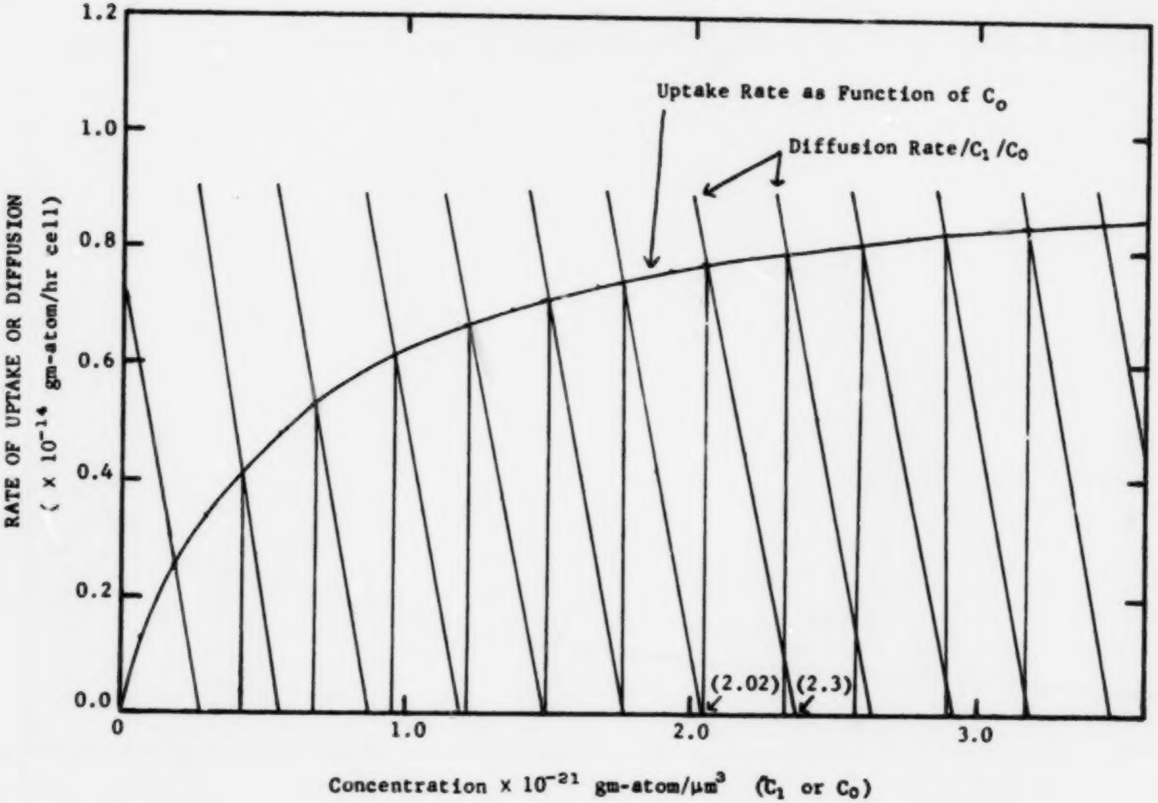

FIGURE 4

CONSTRUCTION DIAGRAM TO MATCH THE RATE OF DIFFUSION INTO A CELL WITH THE RATE OF UPTAKE

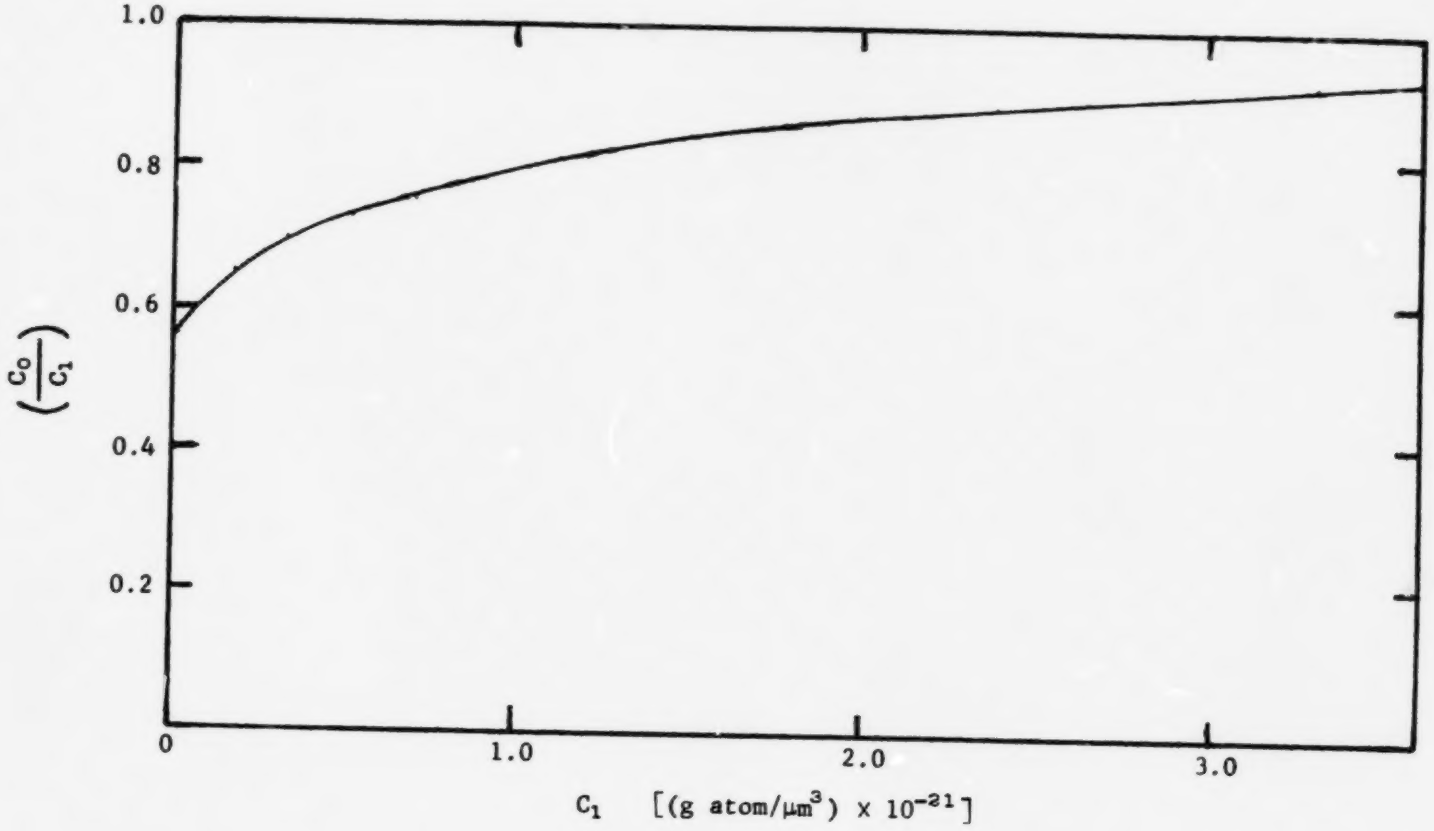

FIGURE 5

RELATIVE CONCENTRATION OF PHOSPHORUS AT THE CELL WALL COMPARED TO BULK CONCENTRATION

A MODEL FOR INTERNAL MASS TRANSFER RESISTANCE TO NUTRIENT UPTAKE IN CELL AGGREGATES

In natural ecosystems, algal cells often aggregate into colonies. In such aggregates, nutrients must diffuse past the outer layer of cells before being taken up by the innermost cells. In modeling nutrient uptake in a colonial system, it is assumed that the aggregate acts as a homogeneous sphere that absorbs nutrients as they diffuse to the center of an effective sphere. It is further assumed that external resistance to movement of material is small compared to internal diffusional resistance. Thirdly, it is assumed that the packing of spherical algae cells within an aggregate is optimal; and thus for a large number of cells within an aggregate, the volume fraction of cells is 0.707.

Including the above assumptions, the following equations describe steady-state uptake of nutrients over a single cell cycle

$$\frac{[D]}{r^2} \frac{d}{dr}\left\{r^2 \frac{d(c)}{dr}\right\} - V_f\ [K]\ (C) = 0\ ,\ r \leqq R_0 \qquad (10)$$

$$(C) = (C_0),\ r = R_0 \qquad (11)$$

$$\left.\frac{\partial (C)}{\partial r}\right|_{r = 0} = 0 \qquad (12)$$

where V_f = volume fraction of algae.

We further assumed that linear kinetics describe the uptake of nutrients, and full coupling of constituents is permitted.

The solution to this set of equations is

$$(C) = \frac{R_0}{r} \sinh\left\{ \{V_f\ [D]^{-1}\ [K]\}^{\frac{1}{2}}\ r \right\}$$

$$\left\{\sinh\left\{ \{V_f\ [D]^{-1}\ [K]\}^{\frac{1}{2}}\ R_0\right\} \right\} \cdot (C_0) \qquad (13)$$

Figure 6 shows this solution for the scalar form of equation (13), which would be applicable to the case of a single limiting nutrient with no diffusional coupling.

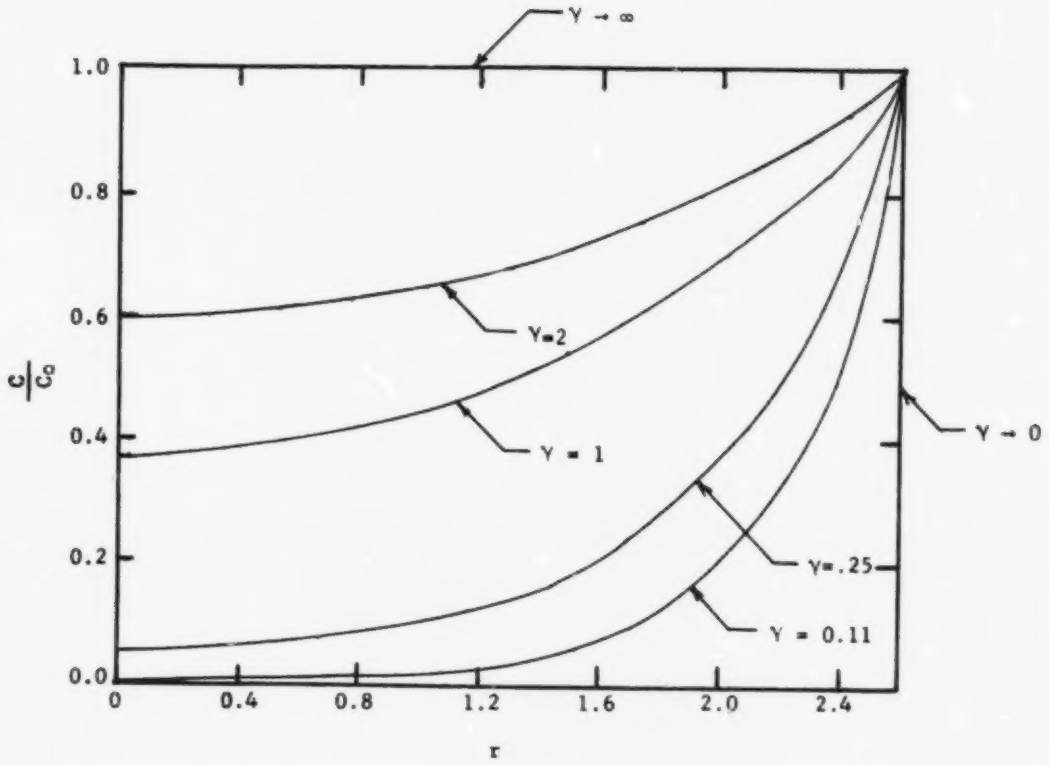

Figure 6

CONCENTRATION PROFILE WITHIN AN ALGAE CELL AS A FUNCTION OF RADIUS AND RELATIVE UPTAKE RATES ($D \cdot K_m/V_m$)

Now, the rate of nutrient uptake at steady-state must be equal to the rate of diffusion of nutrients into the aggregate

$$(R) = \frac{4\pi}{3} R_o^3 \; V_f \; [K] \left\{ \frac{3}{R_o} \left\{\frac{[D]}{V_f} [K]^{-1}\right\}^{\frac{1}{2}} \coth \left\{\{V_f \; [D]^{-1} [K]\}^{\frac{1}{2}} R_o\right\} - 3 \frac{[D][K]^{-1}}{V_f \; R_o^2} \right\} (C_o) \qquad (14)$$

The rate of uptake that would occur if there were no diffusional limitations is simply

$$R = \frac{4}{3} \pi \; R_o^3 \; V_f \; [K] \; (C_o) \qquad (15)$$

Thus the relative rates of uptake can be related to the maximum uptake rate by a matrix of efficiency coefficients, $[\eta]$

$$[\eta] = \frac{3}{R_o V_f} [D][K]^{-1} \left\{R_o \left\{\frac{[D]}{V_f} [K]^{-1}\right\}^{\frac{1}{2}} \coth \left\{\{[D]^{-1} \; V_f \; [K]\}^{\frac{1}{2}} R_o\right\} - [I]\right\} \qquad (16)$$

which is valid for any matrices [D] and [K]. Figure 7 shows the relationship between efficiency and γ, the ratio of D to K, for the scalar case of a single limiting nutrient and a single algal cell of 5.29 μm diameter. If we assume an uptake coefficient, K, for phosphorus of 53.1 $\sec^{-1}$ and a diffusion coefficient, D, of 100 μm^2/sec, γ is equal to 1.88 and the relative uptake rate is approximately 80% of the uptake rate with no diffusion resistance. Table 1 shows the effect of increasing the number of algae cells in a cluster assuming the same properties of the algae aggregate as for the single cell.

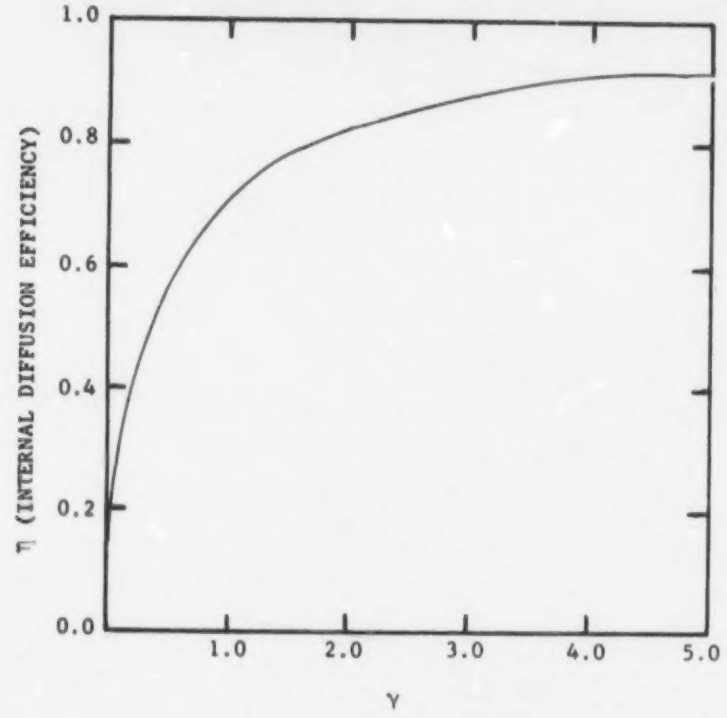

Figure 7

INTERNAL EFFICIENCY AS A FUNCTION OF RELATIVE UPTAKE RATE ($D \cdot K_m/V_m$)

TABLE 1

REDUCTION IN UPTAKE EFFICIENCY WITH INCREASING SIZE OF AGGREGATE

Number of Cells Across the Diameter of a Cluster	η (efficiency)
1	.8
3	.562
5	.411
10	.174

RESULTS AND CONCLUSIONS

Figures 8, 9, 10, and 11 show the growth of C. pyrenoidosa at three concentrations of CO_2 and three shake rates. Higher growth rates are attained at the high shake rates in every case. Table 2 lists the ranges of variances in each experiment. No overlap in variance exists for any of the compared data points, indicating significant statistical difference in any pair of curves.

Table 3 lists the number of doublings at each shake rate divided by the number of doublings obtained at the same CO_2 concentration but at zero shake rate at the end of a 72-hour growth period. A three to four-fold stimulation of biological activity is indicated for cultures grown at a shake rate of 17 rpm versus cultures grown in still vessels. No further stimulation occurred when the shake rates were increased to 33 rpm. Apparently the major boundary layer resistances are eliminated at the lower shake rate.

In interpreting the results of these experiments it is necessary to develop some criterion for quantitating the level of turbulence in the culture. Such a measure is a Reynolds number, which is defined as

$$N_{Re} = \frac{\text{average velocity of shaking} \times \text{a characteristic dimension} \times \text{density of fluid}}{\text{viscosity of fluid}}$$

If an effective dimension of 2.5 cm (the depth of the culture) is used, the Reynolds numbers corresponding to the two shake rates in the experiments are 1930 and 3746 respectively. It would be difficult to extrapolate these data to comparable Reynolds numbers in the field since turbulence is induced by oscillating motion at extremely low Reynolds numbers and since boundary layer thickness is probably more dependent on absolute velocity rather than Reynolds number. However, Fuhs[15] has reported significant differences between phosphorus uptake in shaken laboratory cultures and uptake in lakes at comparable concentration. This observation coupled with our

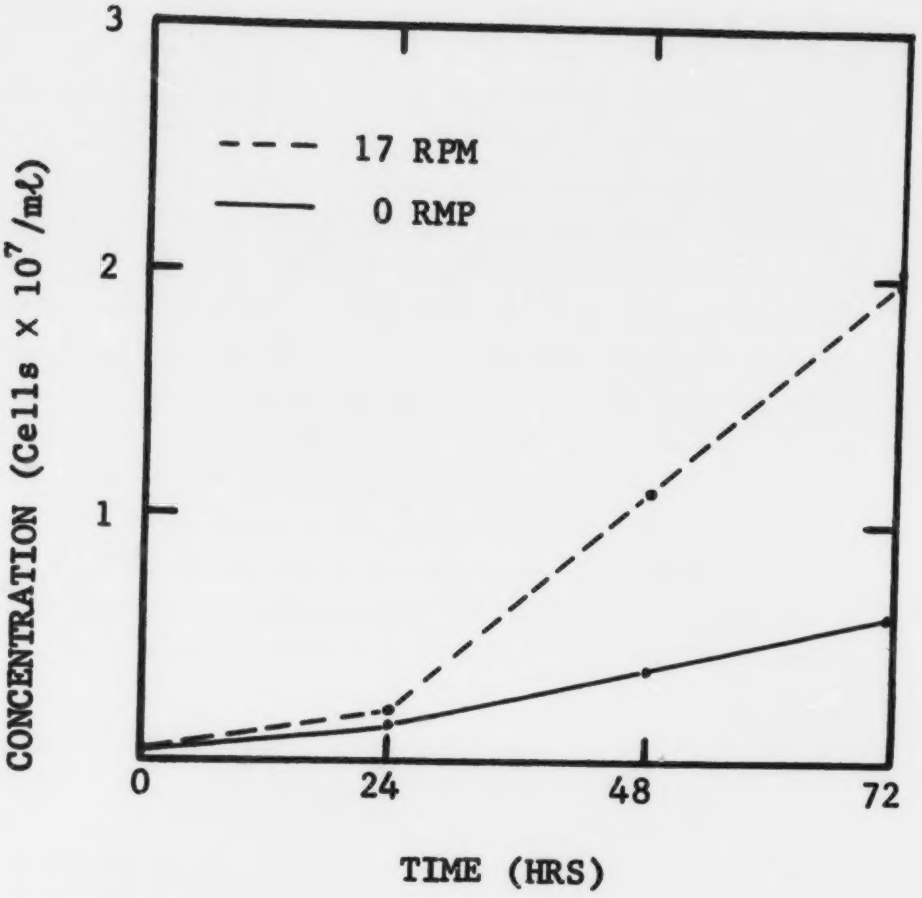

FIGURE 8

CONCENTRATION OF ALGAE AS A FUNCTION OF TIME

(CO_2 CONC = 1%)

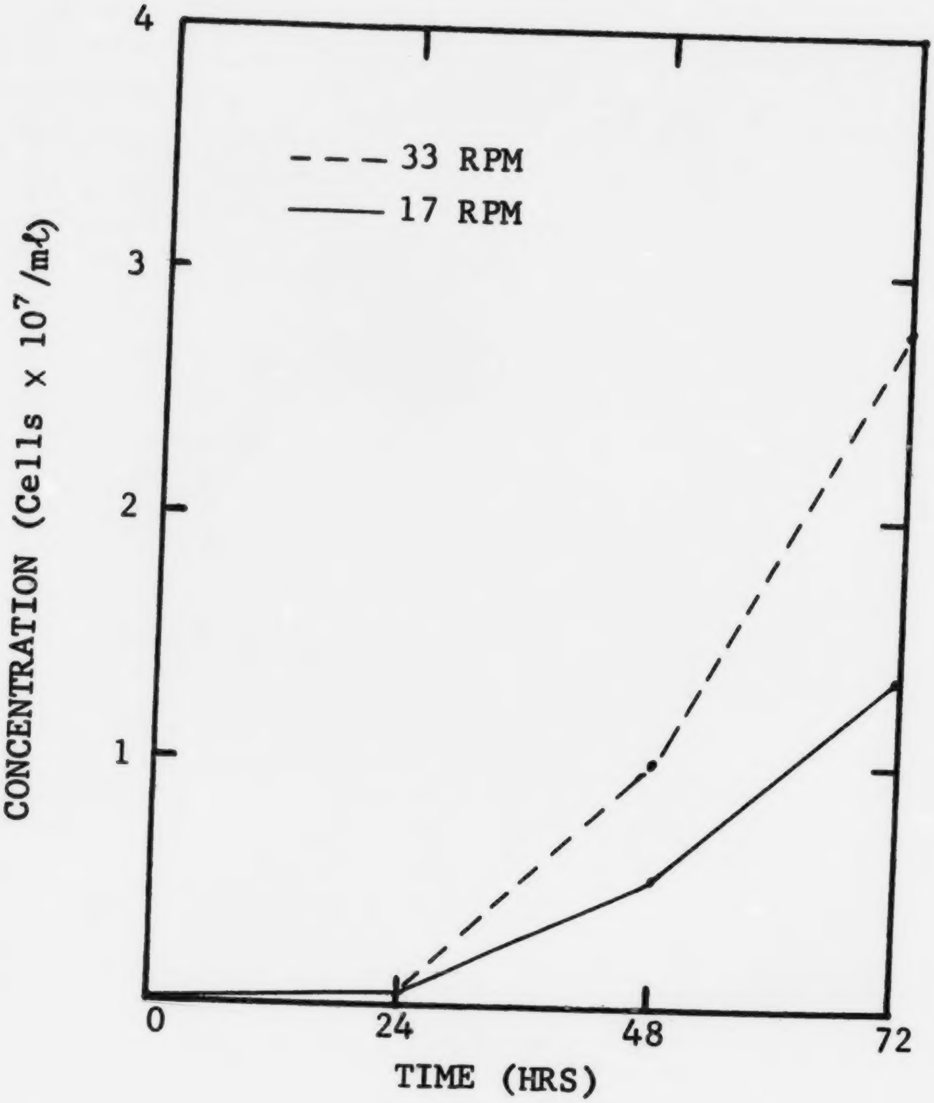

FIGURE 9

CONCENTRATIONS OF ALGAE AS A FUNCTION OF TIME

(CO_2 CONC = 1%)

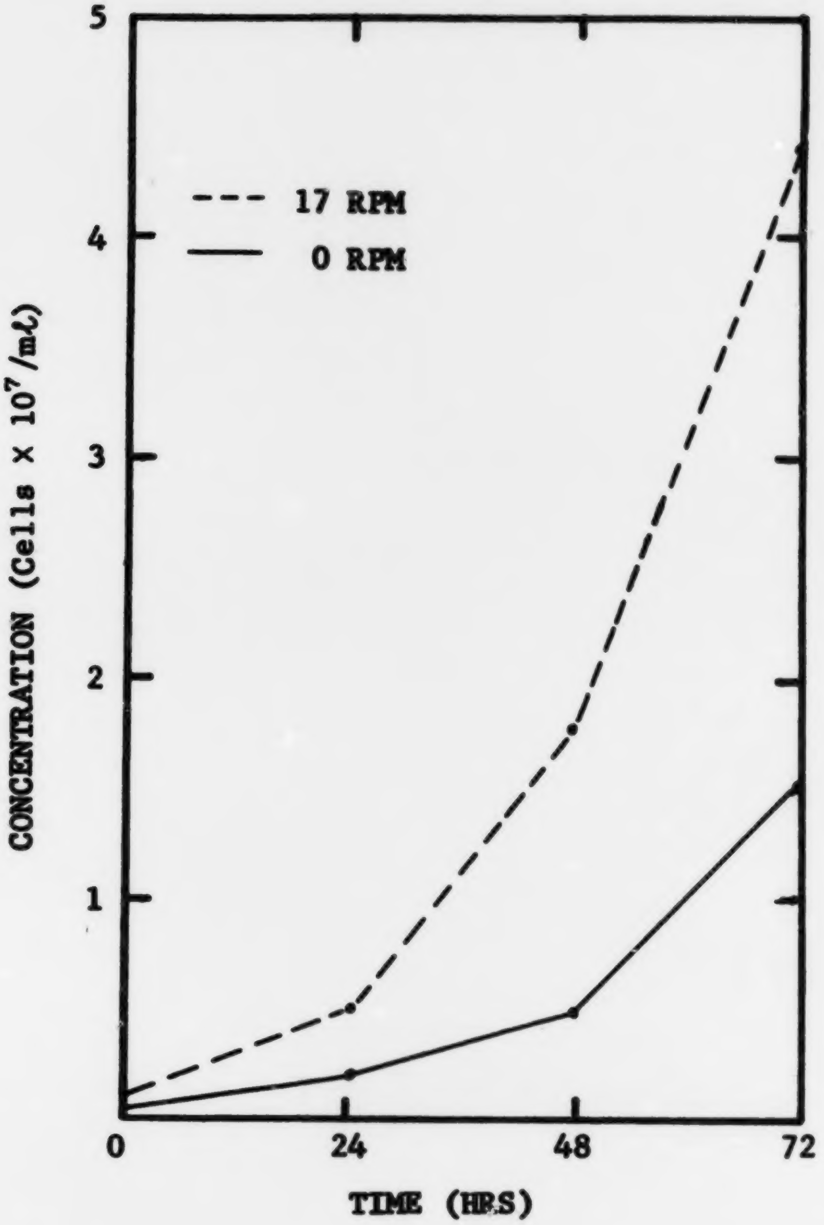

FIGURE 10

CONCENTRATION OF ALGAE AS A FUNCTION OF TIME

(CO_2 CONC = 3%)

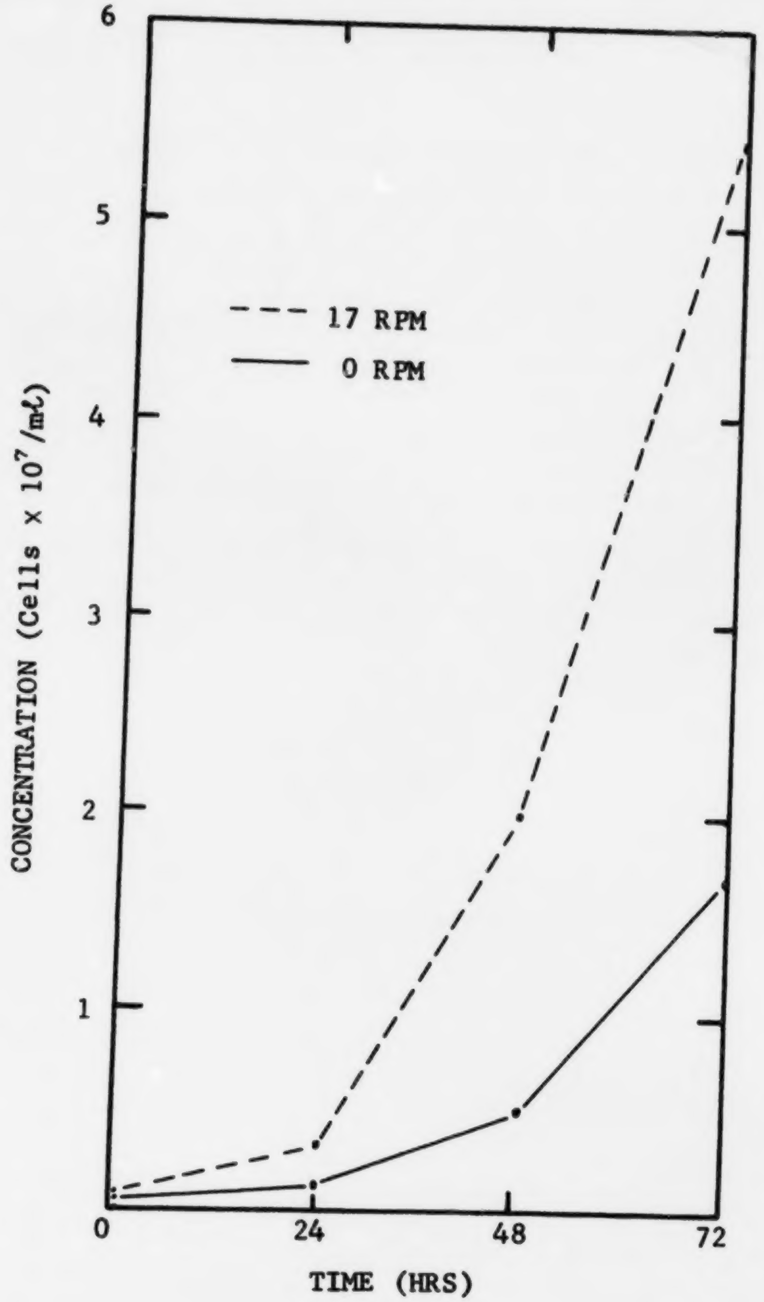

FIGURE 11

CONCENTRATION OF ALGAE AS A FUNCTION OF TIME

(CO_2 CONC = 5%)

TABLE 2

RANGES OF COEFFICIENTS OF VARIATION OF AVERAGED DATA POINTS FOR COMPARATIVE EXPERIMENTS

	Carbon Dioxide Concentration		
Shake Rate (RPM)	1%	3%	5%
0	4-19%	2-32%	5-34%
17	9-31%	20-61%	22-62%

TABLE 3

RELATIVE INCREASE IN ALGAL GROWTH RATE COMPARED TO ZERO SHAKE RATE DURING A 72-HOUR PERIOD

	Carbon Dioxide Concentration		
Shake Rate (RPM)	1%	3%	5%
17	4.2	2.8	3.2
33	4.0		

results indicates that large diffusional resistances can limit uptake of nutrients in natural aquatic ecosystems.

The data collected thus far are insufficient to determine boundary layer thickness and overall mass transfer resistance shown in the mathematical development of the external diffusion resistance model. The limited data indicate that, as predicted by the model, the effect of turbulence on increasing cell growth is less at higher CO_2 concentrations. As more data are obtained at intermediate shake rates and at lower nutrient concentrations, accurate estimates of boundary layer thickness ($R_1 - R_0$) and mass transfer resistance D/K will be obtained.

SUMMARY

A model is proposed to account for mass transport limitation to the uptake of major nutrients. A thin film diffusion resistance layer is hypothesized. In an example using *Cyclotella nana* (a diatom), diffusion resistance was calculated to result in a maximum decrease of 40% in phosphorus uptake rate. Transport limitation was demonstrated experimentally. The growth of *Chlorella pyrenoidosa* (a green alga) was found to be stimulated by shaking the cultures.

Using similar mathematical techniques, the internal resistance to the uptake of nutrients in algal cell aggregates was also modeled.

REFERENCES

1. James, W. O. The Effect of Variations of Carbon Dioxide Supply upon the Rate of Assimilation of Submerged Water Plants. XIX. In: Experimental Researches on Vegetable Assimilation and Respiration. Proc. Roy. Soc. CIII-B:1-42, 1928.

2. Myers, J. The Growth of Chlorella pyrenoidosa under Various Culture Conditions. Plant Physiol. 19:576-589, 1944.

3. Kerr, Pat C., Doris F. Paris, and D. L. Brockway. The Interrelation of Carbon and Phosphorus in Regulating Heterotrophic and Autotrophic Populations in Aquatic Ecosystems. U. S. Environmental Protection Agency, Washington, D. C. 16050 FGS. July 1971. 53p.

4. Borkowski, J. D., and M. J. Johnson. Experimental Evaluation of Liquid Film Resistance in Oxygen Transport to Microbial Cells. Applied Microbiol. 15:1483-1488, 1967.

5. Kalinske, A. A. Effect of Dissolved Oxygen and Substrate Concentration on the Uptake Rate of Microbial Suspensions. Journal WPCF. 43:73-80, 1971.

6. Gulevich, W., C. E. Renn, and J. C. Liebman. Role of Diffusion in Biological Waste Treatment. Env. Sci. Tech. 2:113-119, 1968.

7. Mueller, J. A., W. C. Boyle, and E. N. Lightfoot. Oxygen Diffusion through Zoogloeal Flocs. Biotech. and Bioeng. X: 331-358, 1968.

8. Hartmann, L. Influence of Turbulence on the Activity of Bacterial Slimes. Journal WPCF. 39:958-964, 1967.

9. Whalen, W. J., H. R. Bungay III, and W. M. Sanders III. Microelectrode Determination of Oxygen Profiles in Microbial Slime Systems. Environ. Sci. Technol. 3:1297-1298, December 1969.

10. Jernelov, A., and H. Lann. Studies in Sweden on Feasibility of Some Methods for Restoration of Mercury-Contaminated Bodies of Water. Env. Sci. Tech. 7:712-718, 1973.

11. Toor, H. L. Dual Diffusion-Reaction Coupling in First Order Multicomponent Systems. Chem. Engrg. Sci. 20:941, 1965.

12. Hudson, J. L. Transient Multicomponent Diffusion with Heterogeneous Reactions. Amer. Inst. Chem. Engng. J. 13:961, 1967.

13. Thiele, E. W. Relation between Catalytic Activity and Size of Particle. Ind. Eng. Chem. 31:916, 1939.

14. Wei, J. Intraparticle Diffusion Effects in Complex Systems of First Order Reactions. I. The Effect in Single Particles. Journal of Catalysis. 1:526, 1962.

15. Fuhs, G. W., Susanne D. Demmerle, E. Canelli, and M. Chen. Characterization of Phosphorus-Limited Plankton Algae. In: Nutrients and Eutrophication: The Limiting-Nutrient Controversy, Likens, G. E. (ed.). Lawrence, Allen Press, Inc., 1972. p. 113-133.

TOXICITY OF ZINC TO THE GREEN ALGA SELENASTRUM CAPRICORNUTUM AS A FUNCTION OF PHOSPHORUS OR IONIC STRENGTH

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Tamotsu Shiroyama, and Ellen Merwin

INTRODUCTION

Direct evaluation of waste toxicity through biological assay, involving the use of living organisms as water quality indicators, often is necessary in addition to any chemical examination. Kahn (1964) advocated the use of algae as water quality indicators and suggested the further development of the algal bioassay. In February 1969, a group consisting of government, university and industrial laboratories began research to develop a reliable and reproducible algal assay. As a result of this work, the Algal Assay Procedure Bottle Test (US EPA, 1971) was developed and has been refined sufficiently to be offered now for wider use in connection with eutrophication and other related algal production problems.

The test has been used to determine the effects of secondary and tertiary wastewater effluents in lakes and river systems (Miller, et. al., 1971) as well as to determine growth limiting nutrients in natural waters (Maloney, et. al., 1972). In addition, it has been used to evaluate the effect of various waste treatment processes on the amount of algal growth in treated effluent and to determine growth limiting nutrients in each type of effluent.

The study described herein is concerned with evaluating the ability of the test to identify the potential toxicity of heavy metals in a defined inorganic medium.

METHODS

The Algal Assay Procedure Bottle Test (US EPA, 1971), with modifications, was used in the study. The test alga was *Selenastrum capricornutum* Printz.

Modifications included the following: (1) In addition to the zinc nutrient requirement of the test medium (Table I), zinc spikes were added (as $ZnCl_2$) in increasing 10 μg/l increments. Three replicate flasks containing algal assay medium without zinc additions served as controls and one flask was prepared for each elevated zinc concentration; (2) each flask was inoculated to give a final concentration of 10,000 cells/ml from a seven-day-old culture of _S_. _capricornutum_; (3) assays were carried out in 500 ml polycarbonate Erlenmeyer flasks containing 100 ml of total sample; and (4) all polycarbonate Erlenmeyer flasks and laboratory glassware used for test preparation and algal culture were leached of heavy metal contaminants with a 10 percent solution of nitric acid.

EXPERIMENTAL DESIGN

Zinc-Phosphorus Interactions

The following tests were designed to determine if a relationship existed between zinc and phosphorus concentrations, in a defined inorganic medium, that would affect the 14-day maximum growth yield of _S_. _capricornutum_. One-fourth strength algal assay medium containing 0.047 mg P/l was prepared and dispensed into seven one-liter graduated cylinders. Phosphorus (as K_2HPO_4) was added to each of the seven cylinders to give final concentrations of 0.047, 0.093, 0.139, 0.186, 0.279, 0.372 and 0.465 mg P/l. To another set of eight cylinders, one-half strength medium, also containing 0.047 mg P/l, phosphorus was added to give final concentrations of 0.047, 0.093, 0.139, 0.186, 0.372, 0.558, 0.774 and 0.930 mg P/l. This resulted in 15 distinct media--phosphorus test substrates into which selected concentrations of zinc were added.

Element (μg/1)	AAM Concentration 100%	75%	50%	25%	12.5%
N	4200.000	3150.000	2100.000	1050.000	525.000
P	186.000	139.000	93.000	46.000	23.000
Mg	2904.000	2178.000	1452.000	726.000	363.000
S	1911.000	1433.000	955.000	477.000	238.000
C	2143.000	1607.000	1071.000	535.000	267.000
Ca	1202.000	901.000	601.000	300.000	150.000
Na	11001.000	8250.000	5500.000	2750.000	1375.000
K	469.000	351.000	234.000	117.000	58.000
B	32.460	24.345	16.230	8.115	4.057
Mn	115.374	86.530	57.687	28.843	14.421
Zn	15.691	11.768	7.845	3.922	1.961
Co	0.354	0.265	0.177	0.088	0.044
Cu	0.004	0.003	0.002	0.001	0.000
Mo	2.878	2.158	1.439	0.719	0.359
Fe	33.051	24.788	16.525	8.262	4.131
Cl	6587.000	4941.000	3294.000	1647.000	824.000
EDTA*	300.000	225.000	150.000	75.000	37.000

*Sodium Salt

Table I. Chemical composition of the algal assay culture media.

Zinc-Ionic Strength Interactions

In order to determine what relationship existed between zinc and ionic strength and their combined effect on the 14-day maximum yield of *S. capricornutum*, the following tests were designed. Algal assay medium (100 percent) and four dilutions, resulting in ionic strength of 12.5, 25, 50, and 75 percent, were prepared (Table I). In these media, phosphorus, the primary limiting nutrient, increased proportionately with the increase in ionic strength. Four additional nutrient media were prepared which ranged in ionic strength from 25 to 100 percent of the normal algal assay medium. Each of these media contained 0.047 mg P/l.

DISCUSSION AND RESULTS

Heavy metals are normally found in the environment and some perform critical biological functions. As by-products of various industrial processes, however, these substances are likely to become associated with biological materials in toxic concentrations. The state of chemical combination of a pollutant determines its chemical behavior in the aquatic environment. Ionic species, such as heavy metals, tend to be either held in solution or establish solubility or ion exchange equilibria with suspended materials. The introduction of heavy metals into receiving waters and their effects on the biota depend upon a variety of complex responses governed by several basic factors: (1) nature of the metal; (2) heavy metal concentration; (3) environmental characteristics of the receiving system; (4) presence of other toxicants; and (5) exposure time.

Little is known about the role of zinc in phytoplankton production. Only scant information is available concerning those concentrations which most efficiently stimulate growth and those which are most toxic.

Zinc is an essential microinorganic element required in trace amounts for algal growth (Wiessner, 1962; Walker, 1954). Under conditions of elevated concentration, however, the normal enzyme-zinc mechanisms may be significantly altered, giving rise to toxicity. Antagonistic or synergistic interactions may also alter zinc toxicity. Ernst (1968) reported a negative correlation in *Thlaspi alpestre* spp *calaminare* between intercellular zinc accumulation and the phosphate concentration in the medium. Furthermore, Whitton's (1970) studies on the effects of zinc, copper and lead to Chlorophyta from receiving waters cultured in artifical nutrient medium, suggested that a medium, rich in nutrients such as phosphorus, may lower the toxic effects of heavy metals.

In fourteen bioassays, conducted in 25 percent AAM containing orthophosphorus concentrations ranging from 0.047 to 0.465 mg/l, 40.4 ± 9.9 μg Zn/l were required to produce $\geq$ 95 percent inhibition of the 14-day maximum yield ($\geq$ 95 percent I_{14}) of *S. capricornutum* (Figure I). These data suggest that phosphorus did not significantly alter the level of zinc needed to produce $\geq$ 95 percent I_{14} of the test alga. Furthermore, a twenty-fold increase in orthophosphorus concentration in 50 percent AAM also failed to alter the level of zinc necessary to produce $\geq$ 95 percent I_{14} of the test alga (Figure II). The results of these tests indicate that a statistically significant effect of phosphorus upon zinc's toxicity to *S. capricornutum* does not exist. However, the average concentration of zinc producing $\geq$ 95 percent I_{14} in 25 and 50 percent AAM was shifted from 40.4 to 68.0 μg/l. respectively. This decrease in zinc toxicity, observed during the study of zinc-phosphorus interactions, led us to consider ionic strength as a major factor responsible for the toxicity of zinc upon the growth of *S. capricornutum*.

An important part of the chemical environment of a bioassay procedure is the relative concentration of related ions (i.e., Na, K, Mg, and Ca)

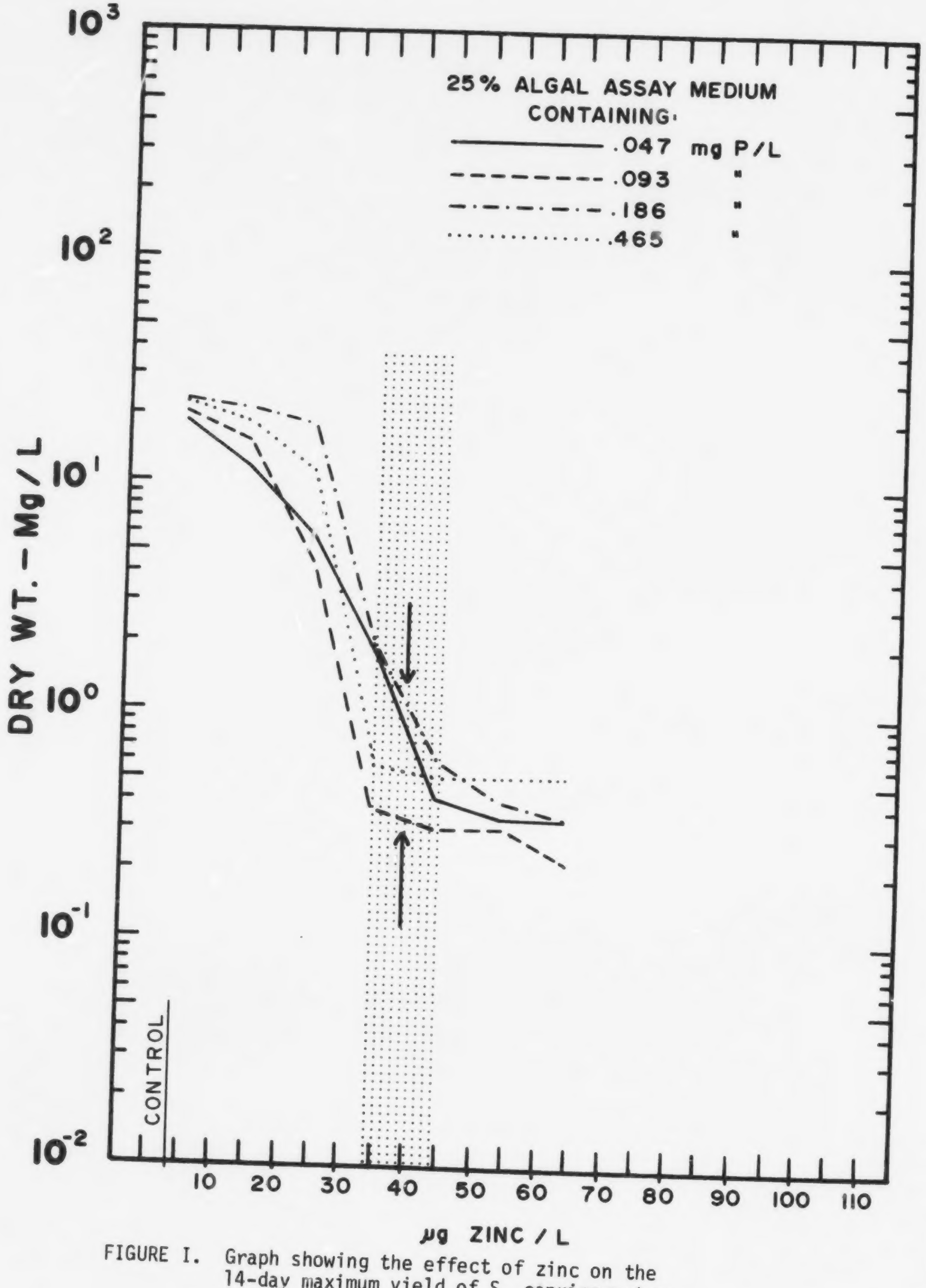


FIGURE I. Graph showing the effect of zinc on the 14-day maximum yield of S. capricornutum cultured in 25% algal assay medium.

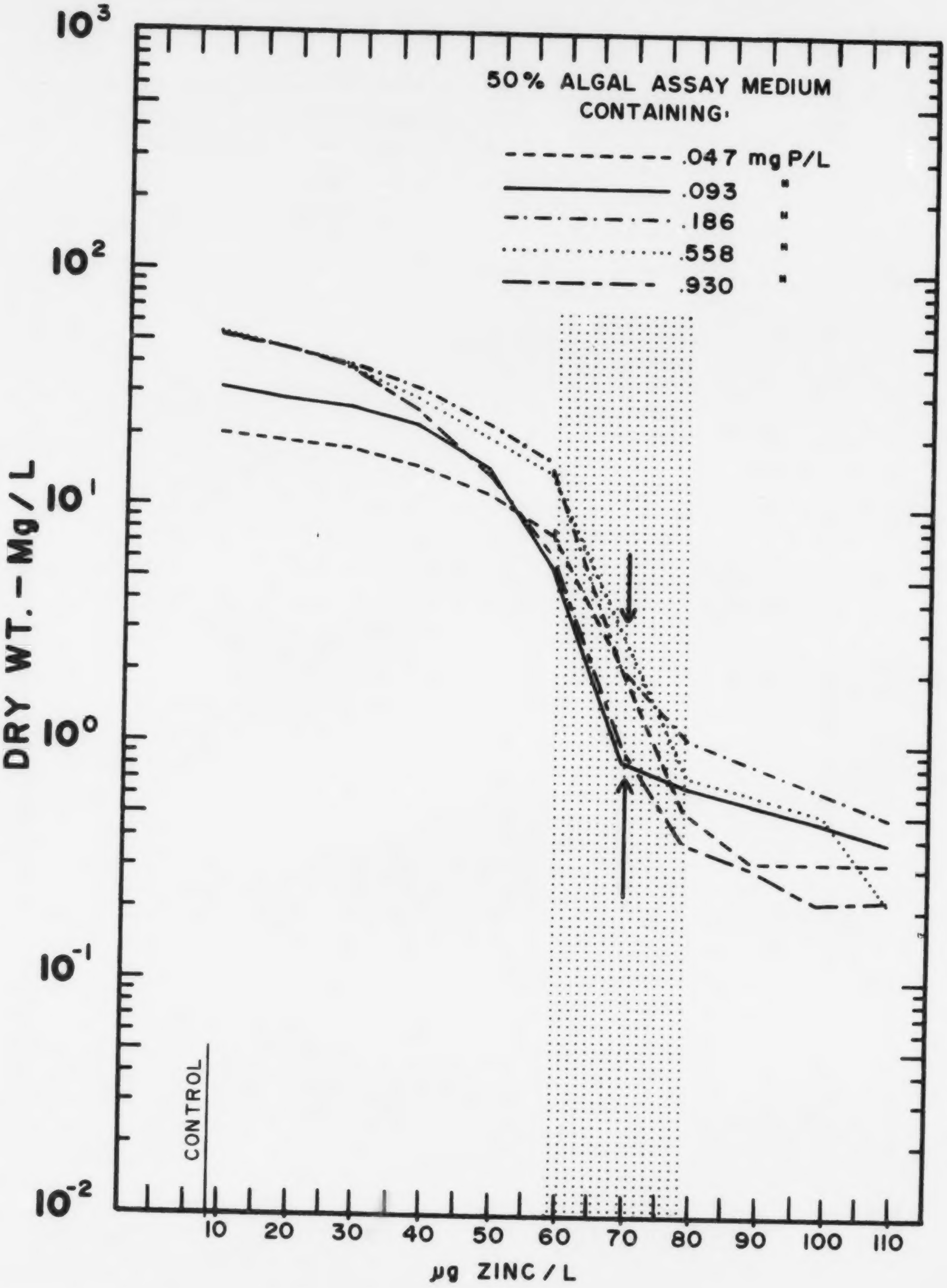


FIGURE II. Graph showing the effect of zinc on the 14-day maximum yield of S. capricornutum cultured in 50% algal assay medium.

present in the medium. This may be in part due to the effect of ionic strength upon the efficiency of solute uptake and perhaps water loss (i.e., osmo-regulation) within the algal cells (Cairns, et al., 1972). In addition to the direct osmotic effects on S. capricornutum, total salts play an important role in influencing the distribution of the chemical species present. Any chemical reactions which are dependent on mass law equilibria will be dependent on the total ionic strength of the solution. Changing the total salt concentration will change the ionic strength which, in turn, decreases the effective concentration of the zinc present in solution. Because heavy metals can be bound to varying degrees by ligands and particles, absolute concentration of a metal does not necessarily mean that amount which has an effect on the test alga. In Figure III the zinc concentration required to achieve similar inhibitory growth effects ($\geq$ 95 percent I_{14}) increased with the increase of specific conductance (ionic content) of the test substrates. Figure IV is a three-dimensional response surface which illustrates the increased requirement for zinc (μg/l) in medium of increasingly greater specific conductance (ionic strength). Normalization of the data shown in Figures III and IV, obtained by dividing the average concentration of zinc required to produce $\geq$ 95 percent I_{14} by the micrograms per liter of total ions in the medium (Figure V), showed that a constant percentage (0.0056) of zinc relative to total ion content was responsible for $\geq$ 95 percent I_{14} in test substrates ranging between 7.9 to 61.0 μmhos/cm conductivity.

The effect of increasing specific conductance can also be seen in Figure VI. However, the maximum algal growth potential in these media was limited by purposely adding only 0.047 mg orthophosphorus/l. This was done to determine if biogenic sites were acting simultaneously with increased specific conductance to decrease the effective concentration of zinc required for $\geq$ 95 percent I_{14} as shown in Figure III. An

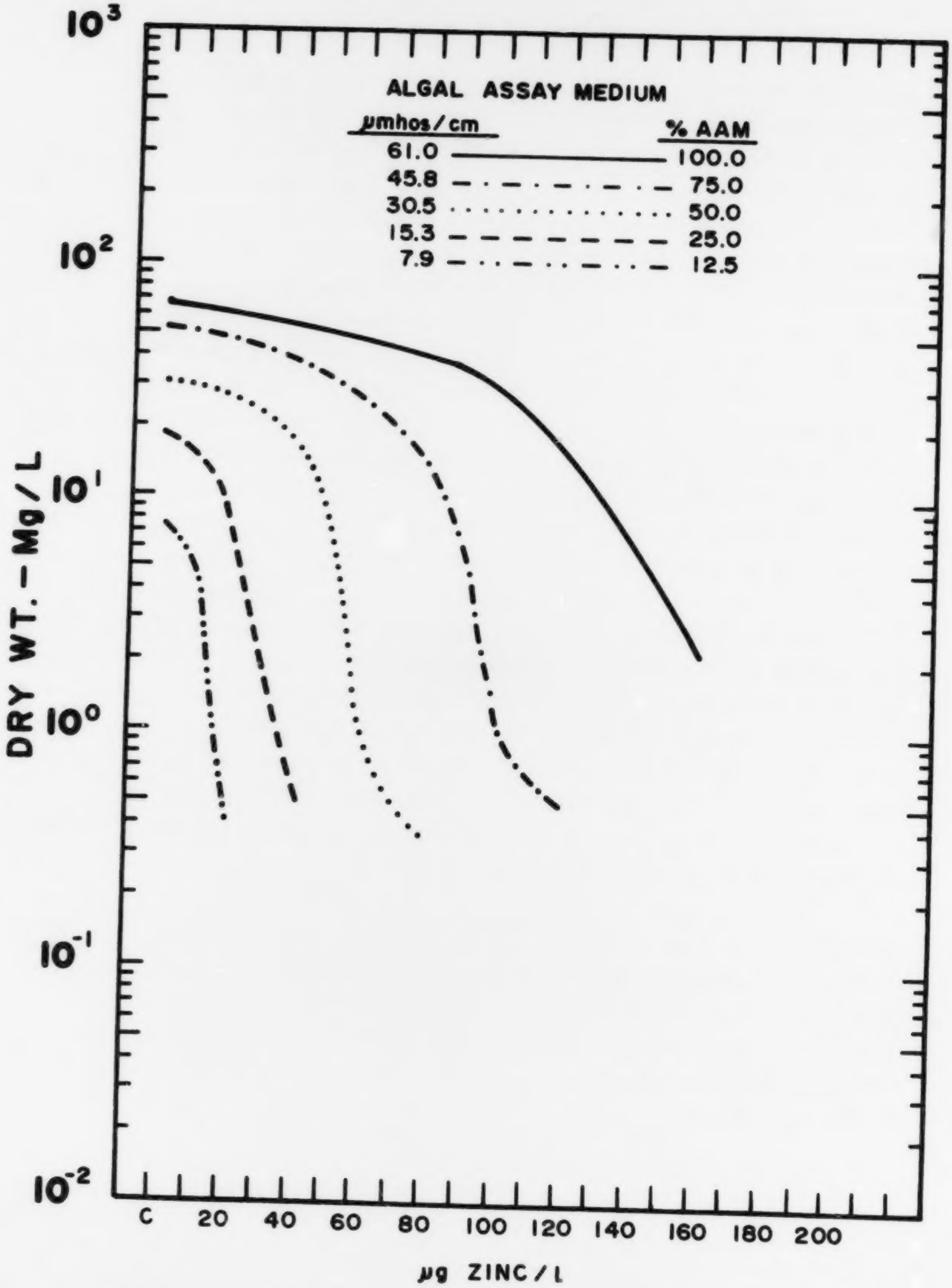
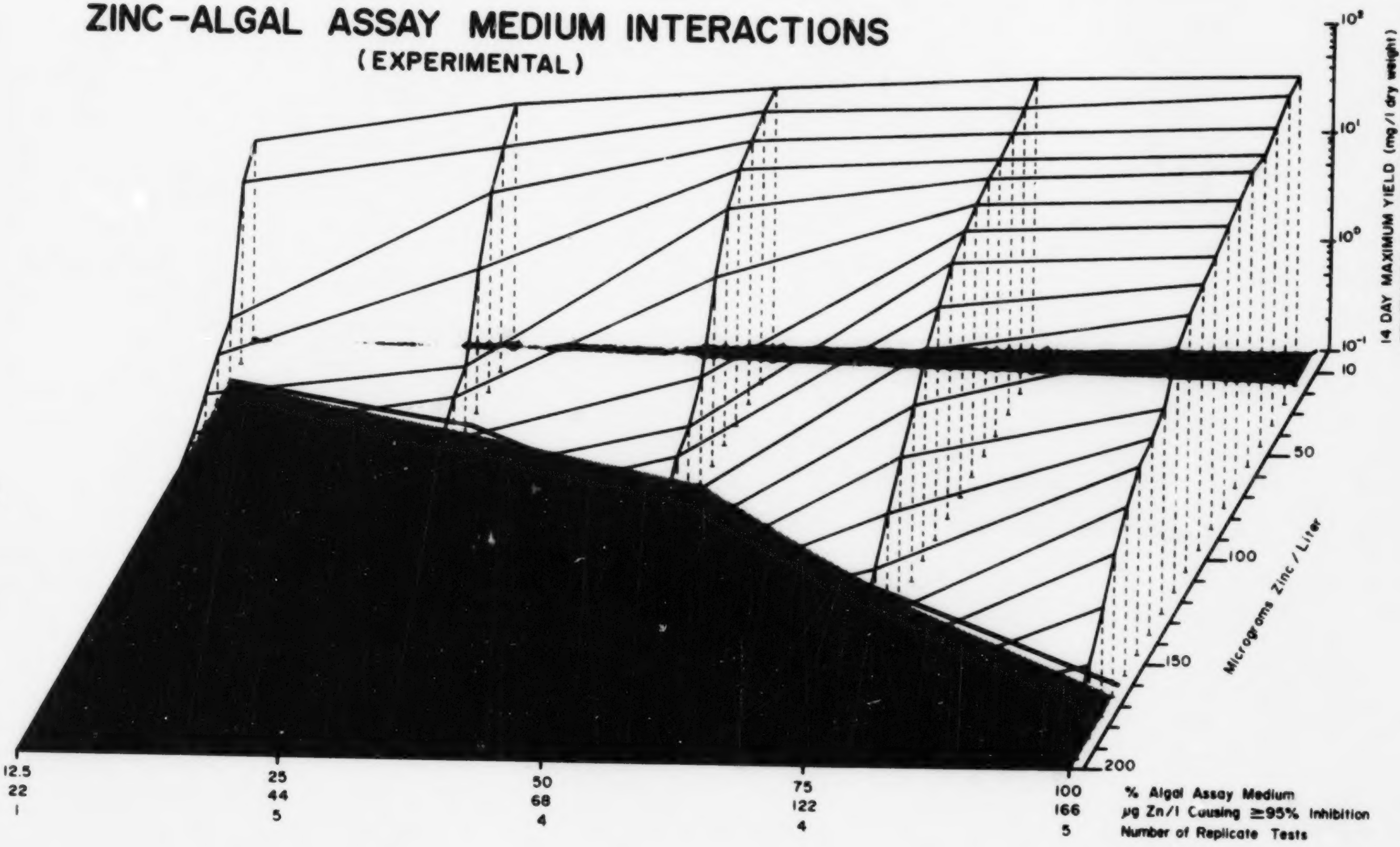


FIGURE III. Graph showing the effects of zinc on the 14-day maximum yield of *S. capricornutum* cultured in media of different total salt concentrations (ionic strength).

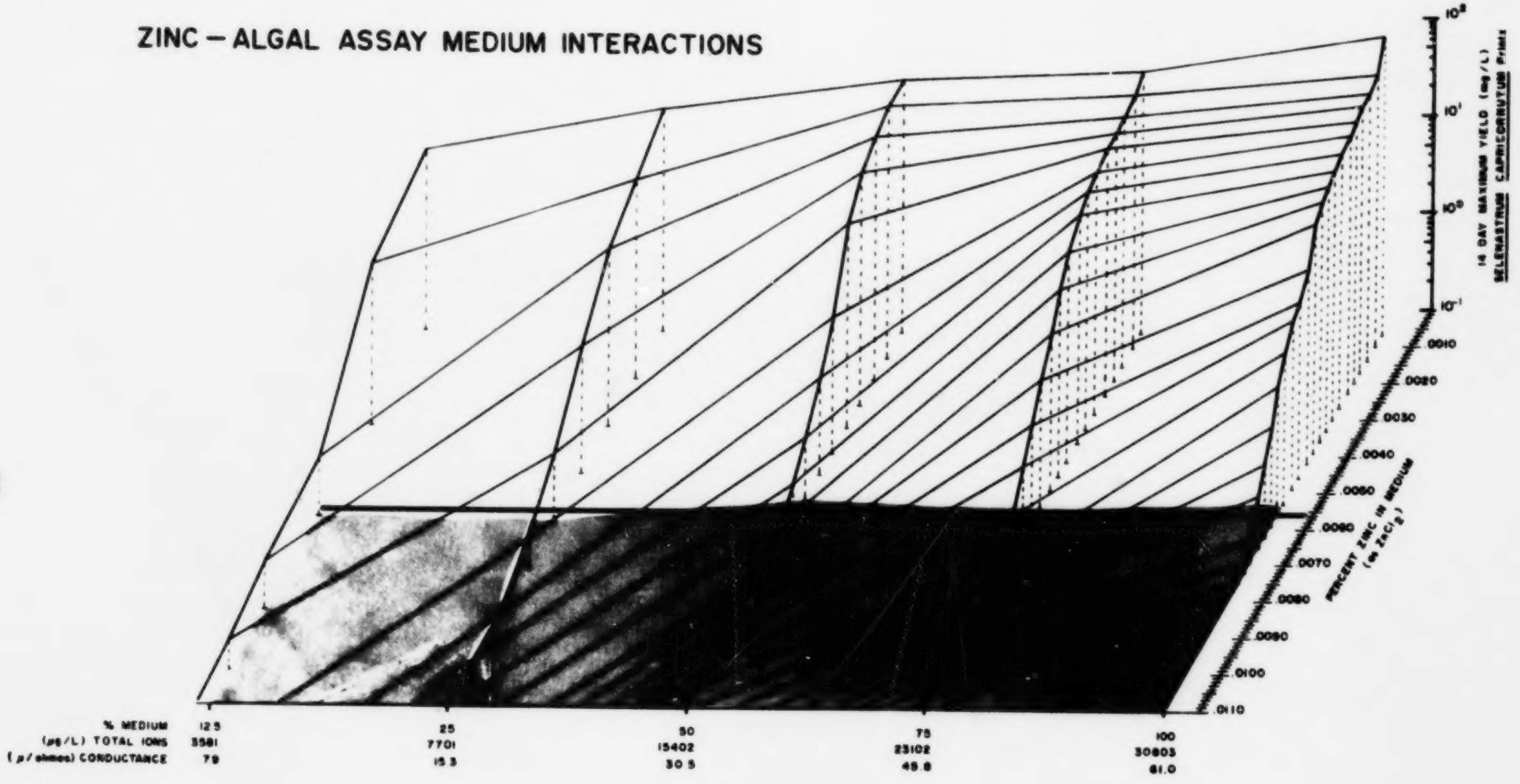


— Represents the theoretical level of zinc (µg/l) that will cause ≥95% inhibition of a 14 day maximum yield of S. capricornutum when compared to the maximum yield produced by the control cultures.

■ Concentration of zinc (µg/l) contained in the algal assay medium.

■ Represents ≥95% inhibition of the 14 day maximum yield of S. capricornutum.

FIGURE IV. Effects of ionic strength and zinc interactions and their combined effect on the maximum yield of S. capricornutum.



▨ ≥ 95 PERCENT REDUCTION OF MAXIMUM YIELD (ALGISTASIS).

ALL CULTURES WERE INOCULATED TO GIVE A FINAL CONCENTRATION OF 10,000 cells/ml.

FIGURE V. Effects of ionic strength and zinc and their combined effect on the 14-day maximum yield of S. capricornutum.

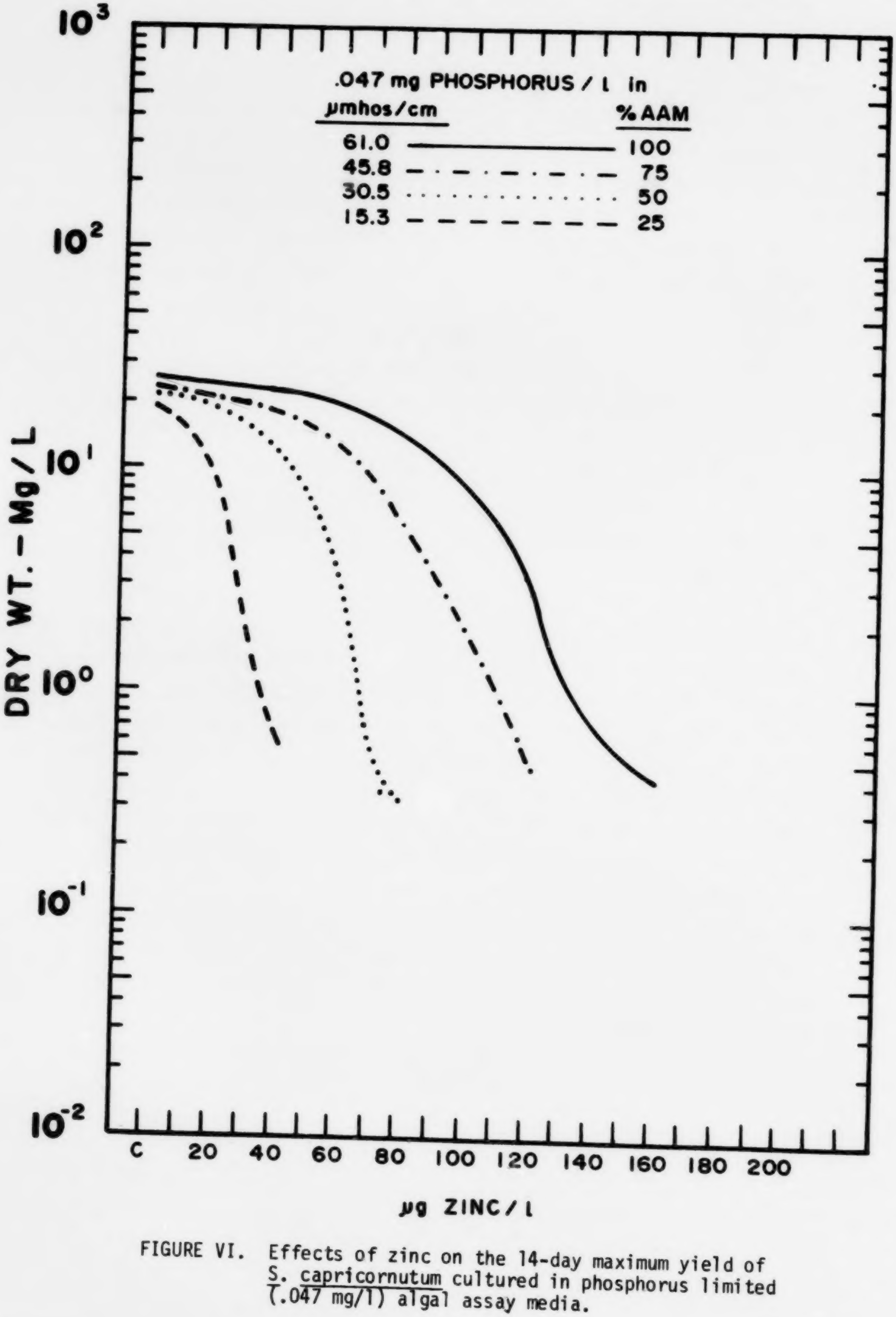


FIGURE VI. Effects of zinc on the 14-day maximum yield of *S. capricornutum* cultured in phosphorus limited (.047 mg/l) algal assay media.

important mechanism of toxic action is thought to be the poisoning of enzymes. Under conditions of elevated concentration the normal enzyme-zinc mechanisms may be significantly altered. In the presence of greater numbers of algal cells (biogenic sites) more enzyme-active sites should be available to tie-up zinc ions. A comparison of Figures III and VI indicate that, within the limits of these tests, there was no significant effect on zinc toxicity caused by the differences in cell numbers.

From the data presented in this paper the ratio of ionic strength as μmhos/cm to the concentration of zinc required to obtain $\geq$ 95 percent algistasis was determined. The ratio (2.72 ± 15 percent) between ionic strength and $\geq$ 95 percent algistatic zinc concentration is significant at the 95 percent confidence level. However, we accept data within ± 20 percent to be statistically valid. The factor of 2.72 ± 20 percent multiplied by the ionic strength (μmhos/cm) of a test substrate should indicate the level of zinc in μg/l that would inhibit $\geq$ 95 percent growth of the test alga provided other antagonistic or synergistic constituents are absent. These data implicate ionic strength as the dominant factor regulating zinc toxicity upon the growth of S. capricornutum. Sensitivity of the test alga to zinc is shown to be inversely proportional to the increase in ionic strength of the test substrate. Ion pair formation may be a significant process by which the availability of zinc ion in waters of higher ionic strength is altered. This may be due to ion pair formation with some of the more common cations present in a receiving water such as calcium, magnesium and sodium. Research is underway to determine if one of these more common cations can be identified as the major constituent causing the decrease of the effective zinc concentration in medium of higher ionic strength.

We recognize that the inability to generalize results from specific observations is a major stumbling block in the use of laboratory developed ecological information for environmental management. We are also aware of the risk of extrapolating from one organism to another and from one geographical locality to another. Hutchinson and Stokes (1973) demonstrated that four species of Chlorophyte alga isolated from metal-contaminated water are tolerant to the heavy metals found in those waters. However, these heavy metals are highly toxic to laboratory strains of the same or closely related species. The greatest scientific benefit will result if intensive research is focused on relatively few kinds of organisms. A large body of information on the physical and chemical requirements of S. capricornutum has been produced. Also, Selenastrum is easy to culture in artificial medium or natural water and, consistently reliable and reproducible growth responses have been obtained with it. For these reasons, we believe S. capricornutum is an excellent test species for the determination of pollutant toxicities.

SUMMARY AND CONCLUSIONS

The Algal Assay Procedure Bottle Test, with S. capricornutum as the algal test species, can be used to identify potential toxicity of heavy metal concentrations in natural waters containing sufficient quantities of nutrients essential for algal growth. Utilization of the AAP Bottle Test indicates that S. capricornutum is highly sensitive to elevated zinc concentrations. The results of the research discussed in this paper indicate:

1. Phosphorus (0.047 to 0.930 mg/l) does not significantly affect the toxicity of zinc to S. capricornutum.

2. Algal cell numbers (biogenic surface area) did not significantly affect the concentration of zinc required to produce $\geq$ 95 percent inhibition of the 14-day maximum yield.

3. Ionic strength is the dominant factor regulating zinc toxicity upon the growth of S. capricornutum. Sensitivity of the test alga to zinc is inversely proportional to the increase in ionic strength (specific conductance) of the test substrates.

4. The factor of 2.72 ± 20 percent multiplied by the ionic strength (μmhos/cm) of a test substrate will indicate the level of zinc in μg/l that would inhibit $\geq$ 95 percent growth of the test alga provided other antagonistic or synergistic constituents are absent.

REFERENCES

Cairns, J., G. R. Lanza and B. C. Parker. 1972. Pollution Related Structural and Functional Changes in Aquatic Communities with Emphasis on Freshwater Algae and Protozoa. Proc. Acad. Nat. Sci. Philadelphia 124(5):79-127.

Ernst, W. 1968. Die Einfluss der Phosphatversorgung sowie die Wirkung von ionogenem und Chelatisiertem Zink auf die Zink - und Phosphatoufnahme einiger Schwermelallpflanzen. Physiol. Plant. 21:323-333.

Hutchison, T. C. and P. M. Stokes. 1973. Metal Toxicity and Algal Bioassays. Symposium on Water Quality Parameters, Canada Centre for Inland Waters, Burlington, Canada.

Kahn, K. R. 1964. Potentiality of Algae in the Bioassay of Microchemical Pollutants in Water Systems. Environ. Health 6:274-277.

Maloney, T. E., W. E. Miller and T. Shiroyama. 1972. Algal Responses to Nutrient Addition in Natural Waters. I. Laboratory Assays. Special Symposia, Volume I, Nutrients and Eutrophication. Amer. Soc. Limnol. and Oceanog.

Miller, W. E. and T. E. Maloney. 1971. Effects of Secondary and Tertiary Wastewater on Algal Growth in a Lake-River System. Jour. Water Poll. Cont. Fed. 33(12).

United States Environmental Protection Agency. 1971. Algal Assay Procedure Bottle Test. National Eutrophication Research Program, Corvallis, Oregon. 82 p.

Walker, J. B. 1954. Inorganic Micronutrient Requirements of Chlorella II. Quantitative Requirements for Iron, Manganese, and Zinc. Arch. Biochem. Biophys. 53:108.

Whitton, B. A. 1970. Toxicity of Zinc, Copper and Lead to Chlorophyta from Flowing Waters. Arch. Mikrobiol. 72:353-360.

Wiessner, W. 1962. Inorganic Micronutrients. In: Physiology and Biochemistry of Algae (R. A. Lewin, ed.). Academic Press, New York. pp. 267-279.

ALGAL ASSAYS FOR THE NATIONAL EUTROPHICATION SURVEY

Albert Katko

INTRODUCTION

The National Eutrophication Survey (NES) was initiated by the U.S. Environmental Protection Agency in 1972 with the primary mission of identifying and investigating those lakes and reservoirs in the contiguous United States which were in danger of eutrophication caused by phosphorus inputs from municipal sewage treatment plant discharges.

In its entirety, the NES effort included sampling of each lake included in the Survey, the tributaries and outlets of each lake, and municipal waste treatment facilities which impacted each lake either by direct discharge to the water body or to a tributary of the water body.

The specific objective, with regard to the approximately 800 lakes eventually to be included in the Survey, is to determine whether nutrient reduction, particularly phosphorus, would significantly improve water quality. To accomplish this the following information must be developed for each lake or reservoir:

1. The present trophic status of the lake and its water quality characteristics.
2. The sources and controllability of nutrients with particular emphasis on nutrients originating from municipal waste treatment facilities.
3. Identification of the nutrient (phosphorus, nitrogen, other) which limits primary productivity.

Upon meeting the objectives and making an evaluation of each lake on a case-by-case basis, joint EPA - State recommendations will be developed for corrective or preventive action.

As of January 1, 1974, sampling programs will be in progress or have been completed in all 27 states east of the Mississippi River involving some 485 lakes and 2,500 stream sampling sites.

To determine the trophic condition of the lakes, the Survey has two helicopters equipped for sampling, each with a team directed by a limnologist. The helicopter teams measure and record the depth, temperature, pH, dissolved oxygen, turbidity, and conductivity with submersible probes. In addition, depth-integrated water samples are taken for chlorophyll and phytoplankton analysis. Three complete circuits of sampling sites are made on each lake during the sampling year.

National Guardsmen sample the significant tributaries to each lake once a month for a year, and sewage treatment plant (STP) effluent samples are collected at the same interval and over the same period by the plant operators.

Algal assays are being conducted to aid in determining the relative trophic status of the study lakes, and to determine the growth limiting nutrient, nitrogen or phosphorus, at the time of sampling.

This report briefly summarizes the procedures used in the Survey algal assays and preliminary findings based on analysis of the first round of data.

METHODS

One set of samples for algal assay is collected by the helicopter team from a lake at a time during the year when the lake is unstratified. Although each lake is sampled three times during the year for other parameters, time and space limitations prohibit performing more than one assay for most lakes. The method for collecting the assay samples is based on the number of stations sampled at each lake. Each assay sample consists of a five gallon composite of one to four depth-integrated samples from the various stations. On large lakes with many stations, one assay sample is composited for each set of up to four stations which are grouped on the basis of the judgment of the field limnologist.

After collection, the lake samples are air-mailed to the laboratory in Corvallis and stored at -23.3° C until the assays can be performed. The samples are prepared as outlined in Figure 1 and according to the procedures described in the Algal Assay Procedure Bottle Test (EPA 1971). The various nutrient additions and the chemical analysis performed on each sample are given in Table 1.

All treatments plus controls are set up in triplicate for each lake water. The test organism is a green alga, *Selenastrum capricornutum*. The cultures are incubated for 14 days at 24 ±2° C under continuous lighting of 400 ft-C on gyratory shakers and growth responses are monitored by periodic cell counts made with an electronic particle counter equipped with a mean cell volume computer. Mean cell volume determinations are converted to equivalent dry-weight values.

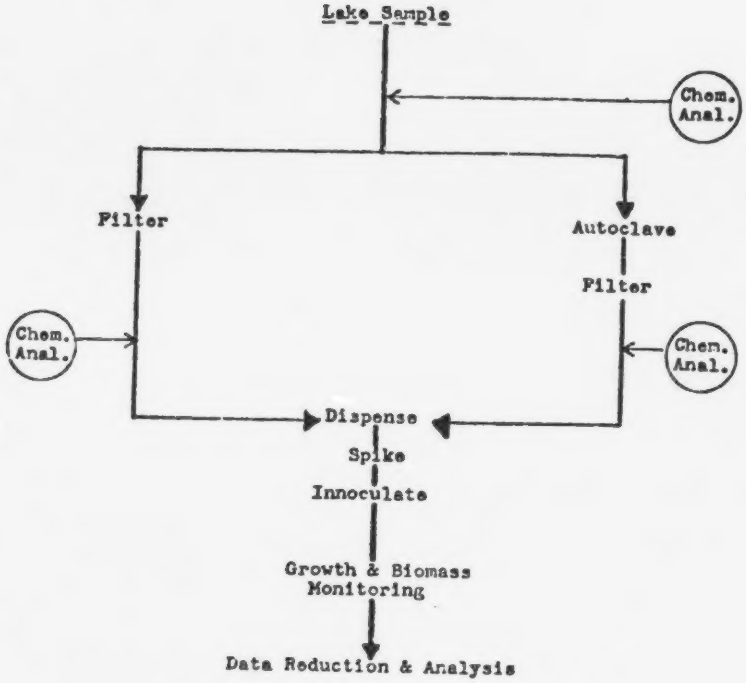

Lake Sample
Chem. Anal.
Filter
Autoclave
Filter
Chem. Anal.
Chem. Anal.
Dispense
Spike
Innoculate
Growth & Biomass Monitoring
Data Reduction & Analysis

Figure 1. Assay flow chart

Table 1

CHEMICAL PARAMETERS & NUTRIENT SPIKES USED IN ASSAY

Chemical Analysis

Conductivity
pH
Inorganic Carbon
Alkalinity
Kjeldahl - N
NH_3 - N
NO_2 & NO_3 - N
Total P
Ortho P
Sulfate
Total Hardness
Calcium
Magnesium
Sodium
Potassium
Chloride
Silicon
Iron
Manganese

Spike Concs. mg/l

Control
1.0 N
0.01 P
0.02 P
0.05 P
0.025 P & 0.5 N
0.05 P & 1.0 N

RESULTS

During the 1972 sampling year, lakes in 10 northeast and northcentral states were sampled, and algal assays were completed on samples from 206 lakes in that area. The productivity range in the control samples (ambient nutrients) from these lakes was very great with yields ranging from less than 0.1 mg/l dry weight to 248 mg/l. Generally speaking, those water bodies which were most enriched were nitrogen-limited while those which had the best water quality were phosphorus limited, although there were exceptions. Occasionally, nitrogen and phosphorus together were required to substantially stimulate a growth response. In a few other cases, algal growth was either limited by the lack of an unidentified constituent or by the presence of toxicant and there was no response to additions of nitrogen, phosphorus, or combinations of the two.

Nutrient limitation for the 206 lakes assayed is summarized in Table 2 by state. As determined by the assay, approximately 57% of all the lakes were P limited, 37% were N limited, 1% were limited by some other element or toxicant, and the limiting nutrient could not be conclusively identified by the remaining 5%. However, the sampling was biased in that the majority of the surveyed lakes received municipal waste effluents.

DISCUSSION

The algal assay procedure is a useful tool in evaluating the trophic condition and the limiting nutrient in a body of water; however, other

TABLE 2

ALGAL ASSAY SUMMARY FOR 1972 NESP LAKES AND RESERVOIRS

State	Number of P Limited Lakes	Number of N Limited Lakes	Number Limited by Another Element	Number for Which Data not Conclusive	Total
Connecticut	3	3	0	2	8
Maine	7	2	0	0	9
Massachusetts	1	7	0	0	8
Michigan	26	8	0	1	35
Minnesota	23	31	2	8	64
New Hampshire	4	0	0	0	4
New York	19	6	0	0	25
Rhode Island	1	1	0	0	2
Vermont	7	0	0	0	7
Wisconsin	26	18	0	0	44
Totals	117	76	2	11	206
% of all	56.9	36.9	1.0	5.2	100.0

biological and chemical parameters should also be examined when the assay results are evaluated for a particular lake.

One of the difficulties, which has not been resolved completely, is how the assay results can be related to actual field conditions and to lake problems or to lake classification. The control yields (representing ambient nutrient levels) should be related to the trophic condition of the lakes, and, in general, they are. A lake which produces a control yield of 1 mg/l or less is generally in acceptable to very good condition, while higher yields indicate conditions ranging from slightly eutrophic to very eutrophic. The highest yield observed in the Survey to data was 248 mg/l, and it is not difficult to interpret such extreme algal assay results; however, the subtle differences are more difficult to interpret. It would be desirable to be able to pinpoint differences in that narrow yield range between oligotrophic and eutrophy which represents but a small segment of the assay yield scale, and also to be able to predict what benefit to a lake, if any, could be expected from nutrient reductions which would result in a change from 20 mg/l yield to 10 mg/l yield. Hopefully, as more assay and field data are assembled by the National Eutrophication Survey and National Eutrophication Research Program, some of these questions will be answered.

Some problems have been encountered in our program during sample transport and treatment which should be noted. Sometimes, significant decreases in conductivity, alkalinity, phosphorus, and nitrogen were observed. The massive logistics of the sampling program make it impossible or economically unfeasible to freeze or ice a large number of samples for shipment, and samples often are in transit from three days to eight days. The preparation procedure, particularly the autoclaved-filtered treatment, occasionally has produced anomalies in the water chemistry. Losses of nitrogen and phosphorus or dissolved minerals have occurred.

Since significant changes in water chemistry can occur during sample transport and processing, it is particularly important to have additional knowledge of the chemistry of each water body at the time of sampling. When these changes and anomalies were observed, little reliance was placed on the assay results.

SUMMARY

The algal assay bottle test has been a useful tool in aiding the evaluation of the trophic state of surface waters and in determining the limiting nutrient for lakes included in the National Eutrophication Survey.

Control yields, in general, have shown a relationship to the trophic state of the lakes in the Survey but must be carefully evaluated in terms of the other parameters and information available.

Thus far the assays have indicated that the majority of the lakes surveyed were phosphorus limited. As more assay data and corresponding field data are collected, and we are better able to relate the meaning of the assay results to field situations, the test will be even more valuable to the Survey.

FREQUENCY ANALYSIS OF CYCLIC PHENOMENA IN FLOWING STREAMS

Pat C. Kerr, James W. Falco, R. Marie Stead,
and Donald Brockway

INTRODUCTION

In an effort to determine those conditions that have the greatest effect on biological activity in a flowing stream, field studies[1,2,3] have been conducted to monitor concentrations of nutrients, including various species of carbon, nitrogen, and phosphorus. Interpretations of temporal and spatial variations in these data, together with supporting laboratory results and theoretical hypotheses, have been used as the basis for both qualitative and mathematical models of flowing aquatic ecosystems.

Most attention to date has been directed toward long-term effects in flowing streams; variables have been averaged over relatively long reaches of streams and over long intervals of time. In contrast, we investigated the cyclic non-random variations in major nutrient concentrations and bacterial populations in oligotrophic first-order mountain streams. Cyclic phenomena in these fast moving streams have three important implications. First, if valid long-term models are to be developed based on field data, sampling periods must be planned so that averaged data reflect the true long-term behavior of the ecosystem rather than short-term fluctuations. Secondly, since water quality and biological activity in these streams are closely coupled to dynamic conditions in the surrounding watershed[4], the parameters that are coupled to watershed conditions and can thus be manipulated by altering land management policy must be determined. Lastly since the long-term fate of major nutrients is directly affected by short-term cycles, the natural frequencies of these variations must be known to adequately describe long-term behavior.

DESCRIPTION OF FIELD SITES

The data investigated in this study were taken during a field study conducted jointly by the U. S. Environmental Protection Agency and the International Biological Program at the U. S. Forest Service Coweeta, North Carolina Hydrologic Station. Since sampling site and procedures have been discussed in a previous report[5], only a brief discussion of the two data sets analyzed will be presented.

Sampling station 6-S is located on a primary stream directly upstream of a weir pond. The watershed surrounding the stream has been perturbed by clear-cutting that was done six years prior to the beginning of the study. Sampling station 18-S is also located on a primary stream directly upstream of a weir pond. The watershed surrounding this stream is a climax eastern deciduous forest. Sampling for parameters shown in Table 1 was done at least once a week, and usually twice a week. The data used in this study was accumulated over 64 weeks of sampling.

Table 1. INDEX OF MONITORED PARAMETERS

pH
Total Organic Carbon (TOC)
Total Inorganic Carbon (TIC)
Carbon Dioxide (CO_2)
Bicarbonate (HCO_3^-)
Total Phosphorus (TP)
Ortho-Phosphorus ($PO_4^{\equiv}$)
Nitrate Nitrogen (NO_3^-)
Dissolved Oxygen (DO)
Sediment Bacteria (Sed. Bact.)
Suspended Bacteria (H_2O Bact.)

FREQUENCY ANALYSIS

The method used to determine major frequencies of cyclic variations in nutrient concentrations was Fourier transformation of the two data sets collected over a 64-week interval starting with the first week of the study. When samples were taken on different days of two consecutive weeks, values were interpolated to obtain estimates of concentrations at even weekly intervals. Since Fourier transforms have not been widely applied to environmental studies, a brief geometric analogy demonstrating the application of Fourier analysis will be given. Most of the material describing Fourier transform has been abstracted from tests by Morse and Feshback[6] and Kreyszig[7].

Consider a plane, i.e., a two dimensional vector space, containing two vectors $\underset{\sim}{A}$ and $\underset{\sim}{B}$, which are 90° apart (Figure 1). Since these vectors are perpendicular, they are linearly independent. This means that, if the length of vector $\underset{\sim}{B}$ is changed, the component of the vector $\underset{\sim}{A} + \underset{\sim}{B}$ in the direction of vector $\underset{\sim}{A}$ remains unchanged. This statement is not true if $\underset{\sim}{A}$ is not perpendicular to $\underset{\sim}{B}$, as illustrated in Figure 2. Two vectors are perpendicular (orthogonal), if their inner product, $\underset{\sim}{A} \cdot \underset{\sim}{B}$, defined by equation (1), is zero.

$$\underset{\sim}{A} \cdot \underset{\sim}{B} = |A|\,|B| \cos(A,B) \qquad (1)$$

Mathematical functions can be treated as vectors in an n-dimensional function space. Addition of vectors in this case is defined simply as arithmetic addition of functions instead of vector addition (as illustrated in Figure 2). An inner product is defined as follows.

If the functions $\underset{\sim}{f}_1(x)$, $\underset{\sim}{f}_2(x)$, ... $f_n(x) \in$ space F, then

$$\underset{\sim}{f}_i \cdot \underset{\sim}{f}_j = \int_0^T w(x)\, f_1(x)\, f_j(x)\, dx \qquad (2)$$

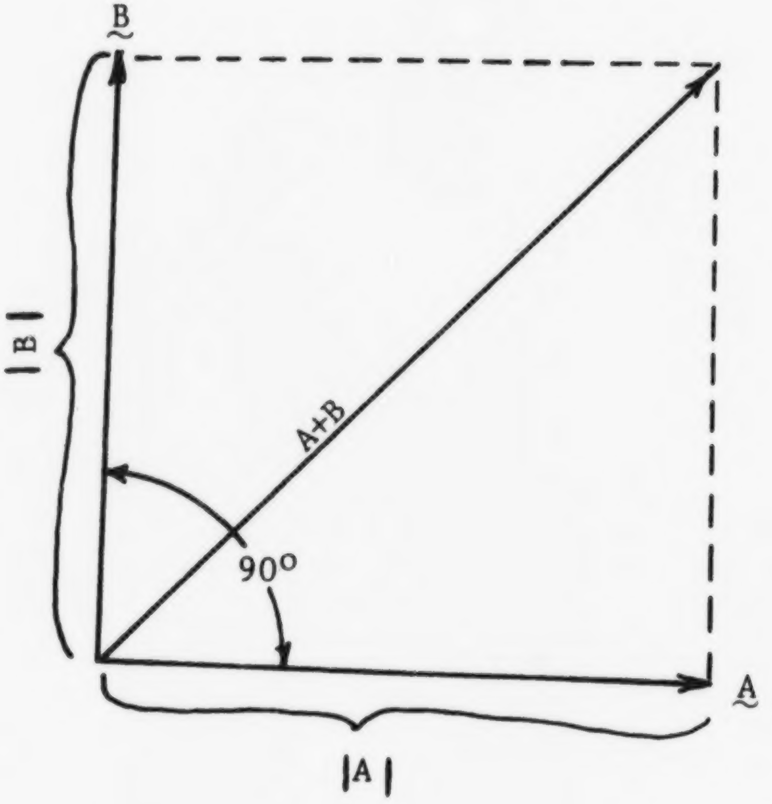

$\underset{\sim}{B}$
$|B|$
A+B
90°
$\underset{\sim}{A}$
$|A|$

Figure 1

ILLUSTRATION OF TWO PERPENDICULAR VECTORS
AND VECTOR ADDITIONS

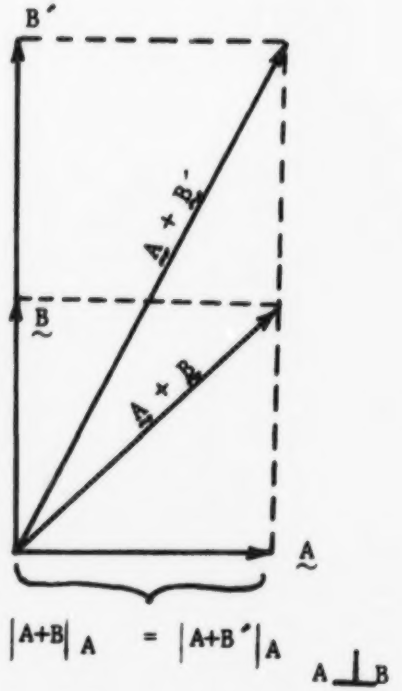

B′
B
A + B′
A + B
A
|A+B|A = |A+B′|A
A ⊥ B

Figure 2a

ILLUSTRATION OF INDEPENDANCE
OF PERPENDICULAR VECTORS

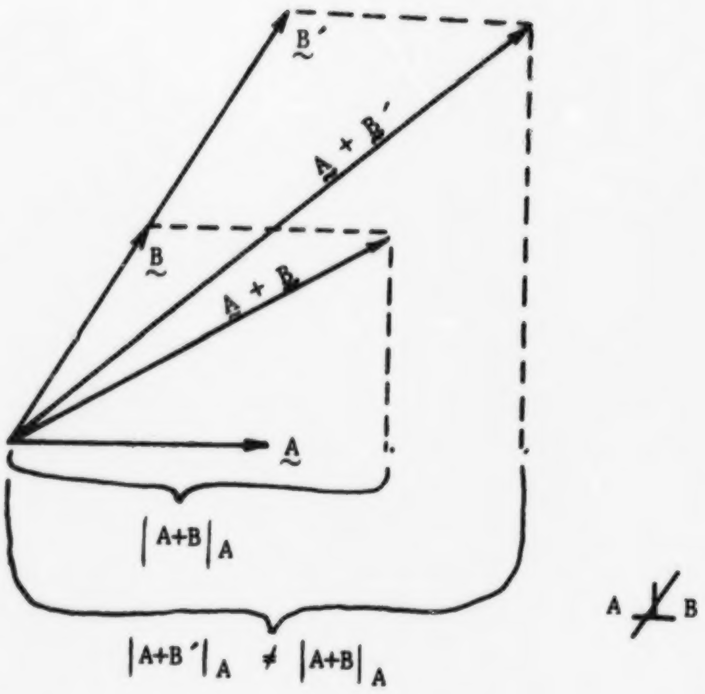

B′
B
A + B′
A + B
A
|A+B|A
|A+B′|A ≠ |A+B|A
A ⊥̸ B

Figure 2b

ILLUSTRATION OF DEPENDANCE OF VECTORS
WHICH ARE NOT PERPENDICULAR

where $w(x)$ = a weight function

T = constant interval of integration

Two functions are orthogonal if their inner product is zero.

$$f_i \cdot f_j = 0 \qquad (3)$$

Now, just as any vector in a two dimensional vector space can be written as the vector addition of two perpendicular vectors that span the space, any function in an n-dimensional function space can be written as the sum of n orthogonal functions that span the function space. More precisely, if $f(x)$ is in a function space spanned by $f_1(x)$, $f_2(x)$, ... $f_n(x)$, then

$$f(x) = \sum_{i=1}^{n} A_i f_i(x) \qquad (4)$$

where all A_i are constants.

In treating non-cyclic phenomena, collected data are usually fit to an n^{th} order polynomial

$$f(x) = A + Bx + Cx^2 + \ldots + Dx^n \qquad (5)$$

that is presumed to describe the process. In terms of the function space concept, data are fit to a function in some n-dimensional polynomial space spanned by a set of n orthogonal polynomials. Often $f(x)$ is then broken up, i.e., decomposed into its orthogonal components.

For cyclic phenomena, the data are fit into a Fourier series of sine and cosine functions

$$f(x) = \sum_{k=0}^{n} A_k \cos(k \pi x) + \sum_{k=1}^{n} B_k \sin(k \pi x) \qquad (6)$$

In this case $f(x)$ is a function in a function space spanned by 1, $\cos(\pi x)$, $\cos(2\pi x)$, ... $\cos(n\pi x)$, $\sin(\pi x)$, ... $\sin(n\pi x)$. It is easy to define an inner product for which these functions are orthogonal. If, in equation (2),

$$\begin{aligned} w(x) &= 1 \\ T &= 1 \end{aligned} \qquad (7)$$

The above trignometric functions have the following properties

$$\int_0^1 \cos (k\pi x) \sin (k\pi x) \, dx = 0 \tag{8}$$

$$\int_0^1 \cos (k\pi x) \sin (m\pi x) \, dx = 0 \tag{9}$$

$$\int_0^1 \cos (k\pi x) \cos (m\pi x) \, dx = 0 \tag{10}$$

$$\int_0^1 \sin (k\pi x) \sin (m\pi x) \, dx = 0 \tag{11}$$

$k \neq m$

Thus $\sin (k\pi x)$ and $\cos (k\pi x)$, for $k = 1, 2, \ldots n$, are a set of orthogonal functions that span a $2n$ dimensional function space. Equations (8) through (11) are valid only when k and m are integers. Finally

$$\int_0^1 \sin^2 k\pi x \, dx = \int_0^1 \cos^2 k\pi x \, dx = \frac{1}{2} \tag{12}$$

A Fourier series (equation 6) may be fit to a data set using values for A_k and B_k, estimated by performing the following integrations:

$$A_k = 2 \int_0^1 f(x) \cos (k\pi x) \, dx = \underline{f}(x) \cdot \underline{f}_k \tag{13}$$

$$B_k = 2 \int_0^1 f(x) \sin (k\pi x) \, dx = \underline{f}(x) \cdot \underline{f}_{2k} \tag{14}$$

which follows from equation (6) and equations (8) through (12).

Fourier transforms are extensions of Fourier series used when large numbers of frequencies are involved. In this case the summation in equation (6) becomes an integral

$$f(x) = A_o + \frac{1}{\pi} \int_0^\infty [A(k\pi) \cos (k\pi x) + B(k\pi) \sin (k\pi n)] \cdot d(k\pi) \tag{15}$$

and equations (13) and (14) become

$$A(k\pi) = \int_{-\infty}^{\infty} f(x) \cos (k\pi x) \, dx \tag{16}$$

$$B(k\pi) = \int_{-\infty}^{\infty} f(x) \sin (k\pi x) \, dx \tag{17}$$

A_o in equation (15) is interpreted as the mean value of the data set. Substituting

$$\sin x = \frac{1}{2i} (e^{ix} - e^{-ix}) \tag{18}$$

$$\cos x = \frac{1}{2} (e^{ix} - e^{-ix}) \tag{19}$$

where

$$i = \sqrt{-1} \tag{20}$$

into equation (15), one obtains another form of the Fourier transform:

$$f(x) = \frac{1}{2\pi} \int_{-\infty}^{\infty} A(w) e^{iwx} \, dx \tag{21}$$

where $w = 2\pi k$.

Both forms of the Fourier transform appear frequently in the literature and should be recognized as equivalent. Since in this preliminary analysis only the magnitude of variation in the parameters was required, absolute values of $A(w)$ and $B(w)$ were added to give the total amplitude of variation at a frequency disregarding phase angle. Throughout the remainder of the paper, these values are referred to as Fourier coefficients.

RESULTS

A computer program that performs fast Fourier transforms, developed by Cooley and Tukey[8], was used to analyze data taken at Coweeta, stations 6-S and 18-S. Since data were taken at weekly intervals, the highest frequency that could be detected was 26 cycles per year. The total analysis time period of 64 weeks was chosen to insure the detection of any annual cycles.

Figures 3-13 show variations in Fourier coefficients as functions of frequency for various parameters at station 6-S. A large annual cycle is evident in each case, with the notable exceptions of total organic carbon and suspended bacteria. These annual cycles are the dominant frequency for dissolved oxygen, nitrate-nitrogen, total phosphorus, and ortho-phosphate. The annual frequency is the only significant nitrate-nitrogen frequency.

Figures 14-23 show variations in Fourier coefficients as functions of frequency for various parameters at station 18-S. Again in all cases except suspended bacteria, a large annual cycle is present. At station 18-S, carbon dioxide, bicarbonate, total phosphorus, and ortho-phosphate exhibit dominant annual cycles.

DISCUSSION AND CONCLUSIONS

Tables 2 and 3 show a summary of major frequencies in parameters measured at stations 6-S and 18-S respectively. Significant nonrandom fluctuations occur in major nutrients and bacterial concentrations in both streams. Both tables also indicate that

- total inorganic carbon, total organic carbon, total phosphorus, suspended bacteria and sediment bacteria exhibit cyclic variations at high frequencies, and
- nitrate-nitrogen exhibited significant cyclic variations only at low frequencies.

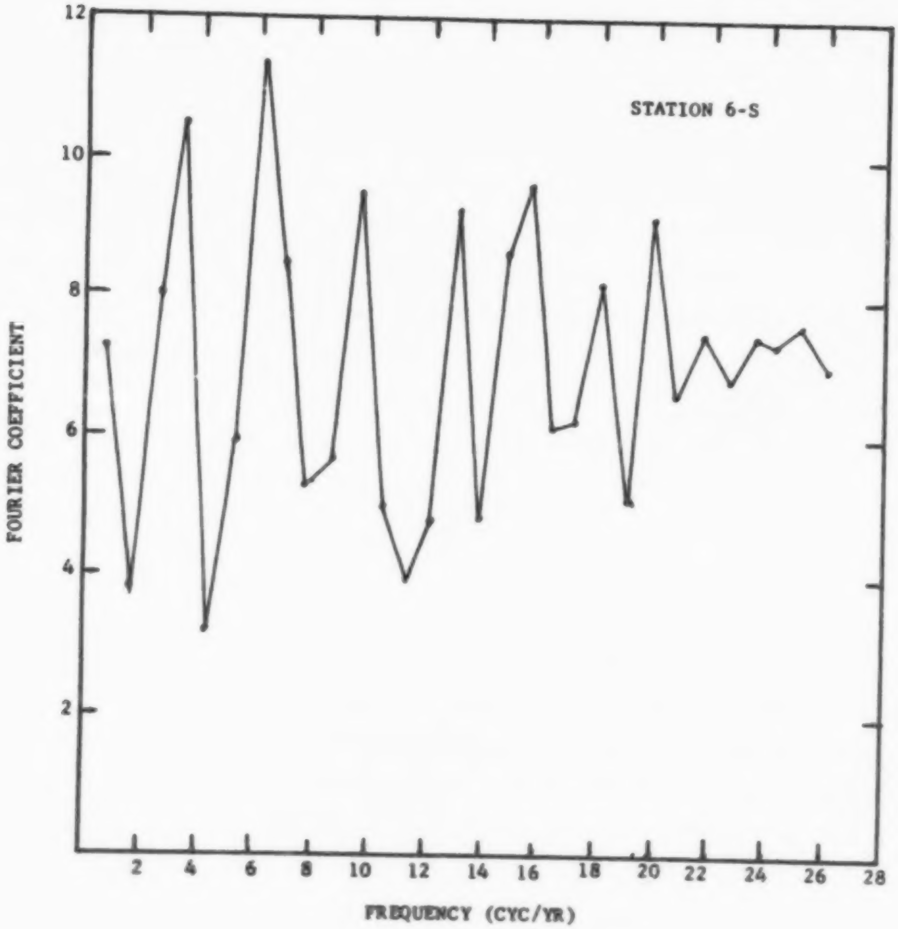


Figure 3

FOURIER TRANSFORM FOR pH

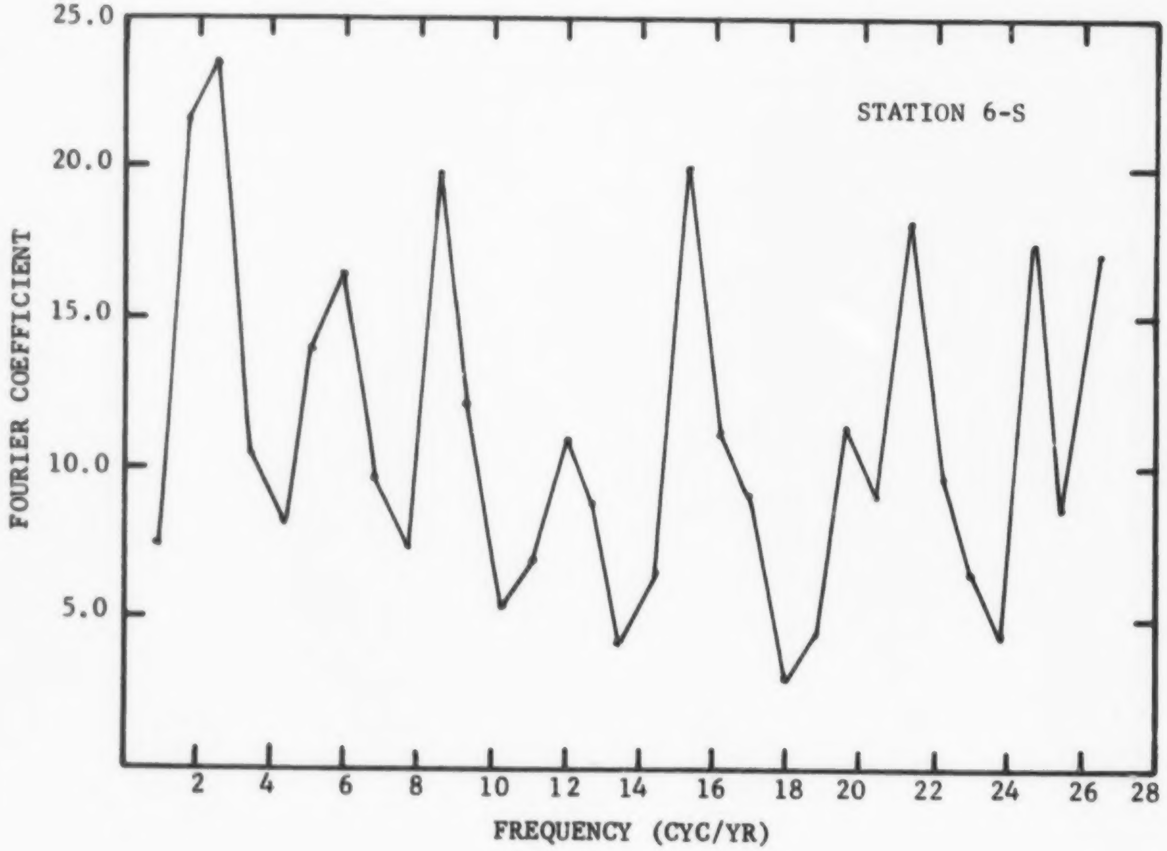


Figure 4

FOURIER TRANSFORM FOR TOTAL ORGANIC CARBON

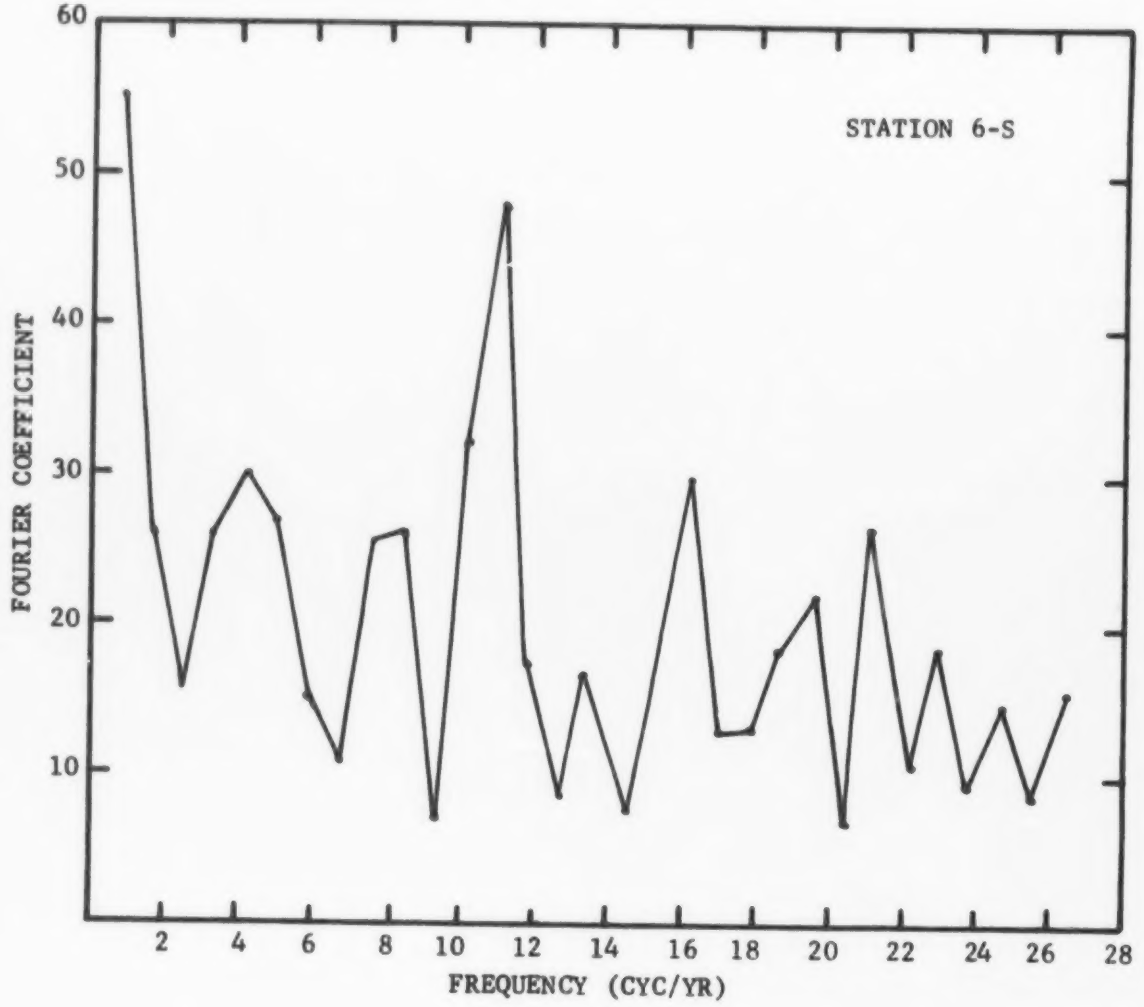


Figure 5

FOURIER TRANSFORM FOR TOTAL INORGANIC CARBON

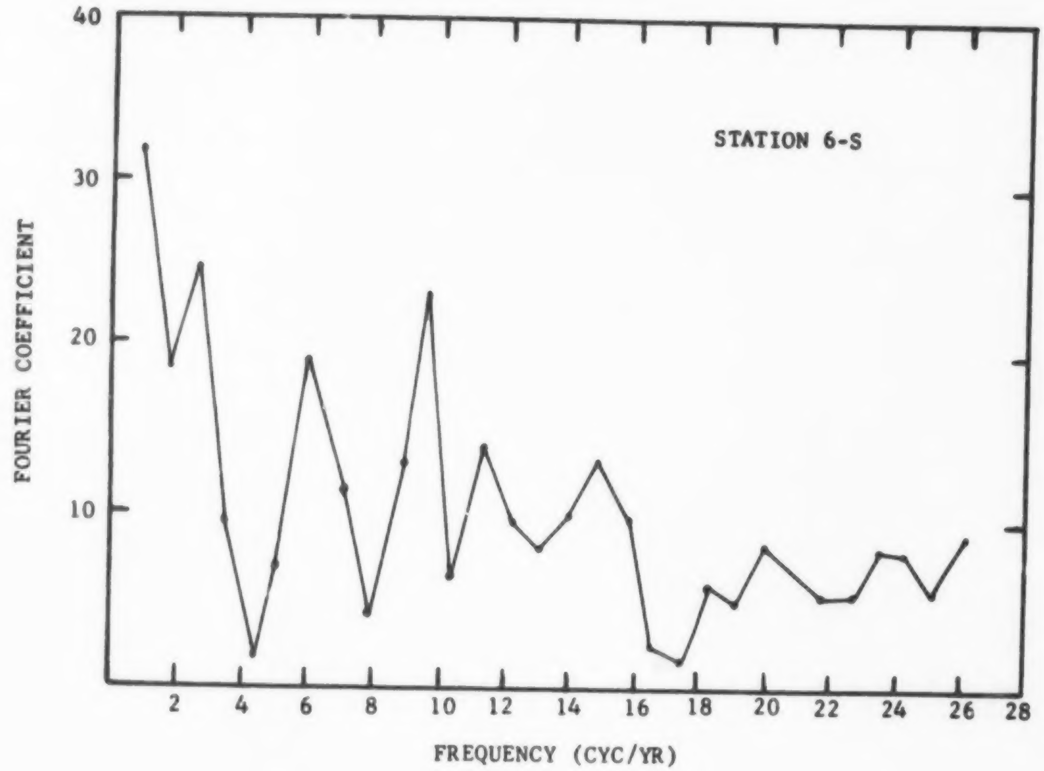


Figure 6

FOURIER TRANSFORM FOR CARBON DIOXIDE

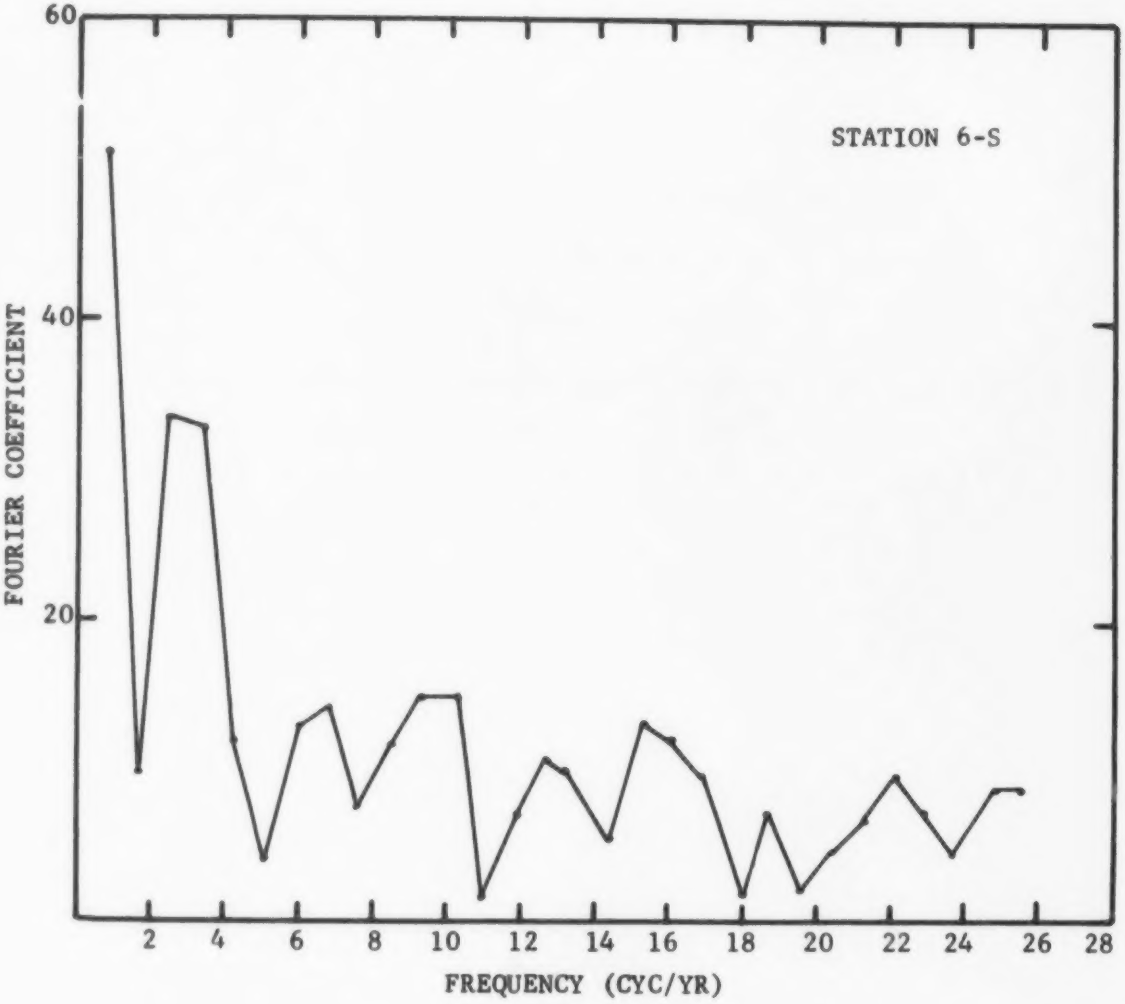


Figure 7

FOURIER TRANSFORM FOR BICARBONATE

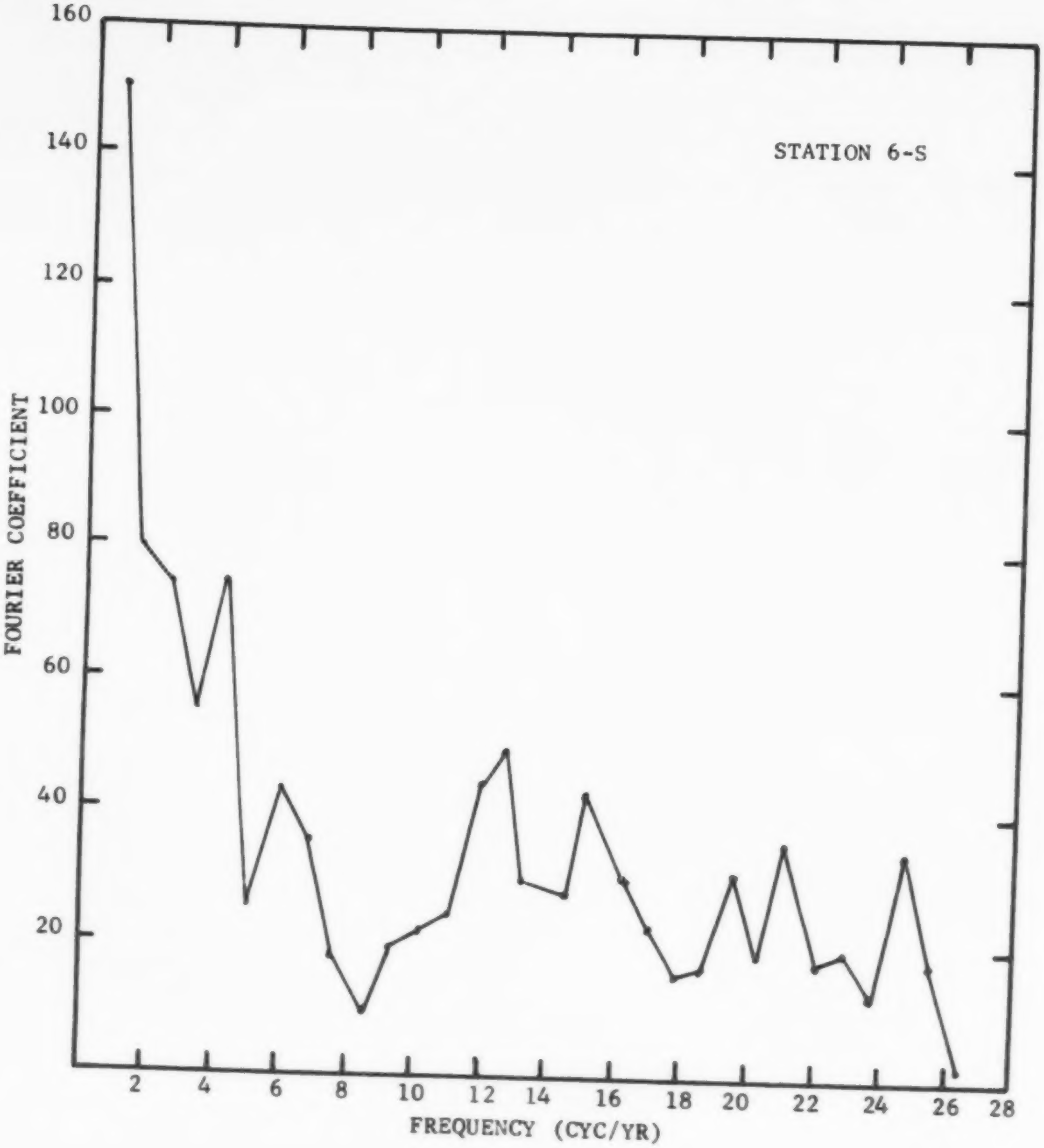


Figure 8

FOURIER TRANSFORM FOR TOTAL PHOSPHORUS

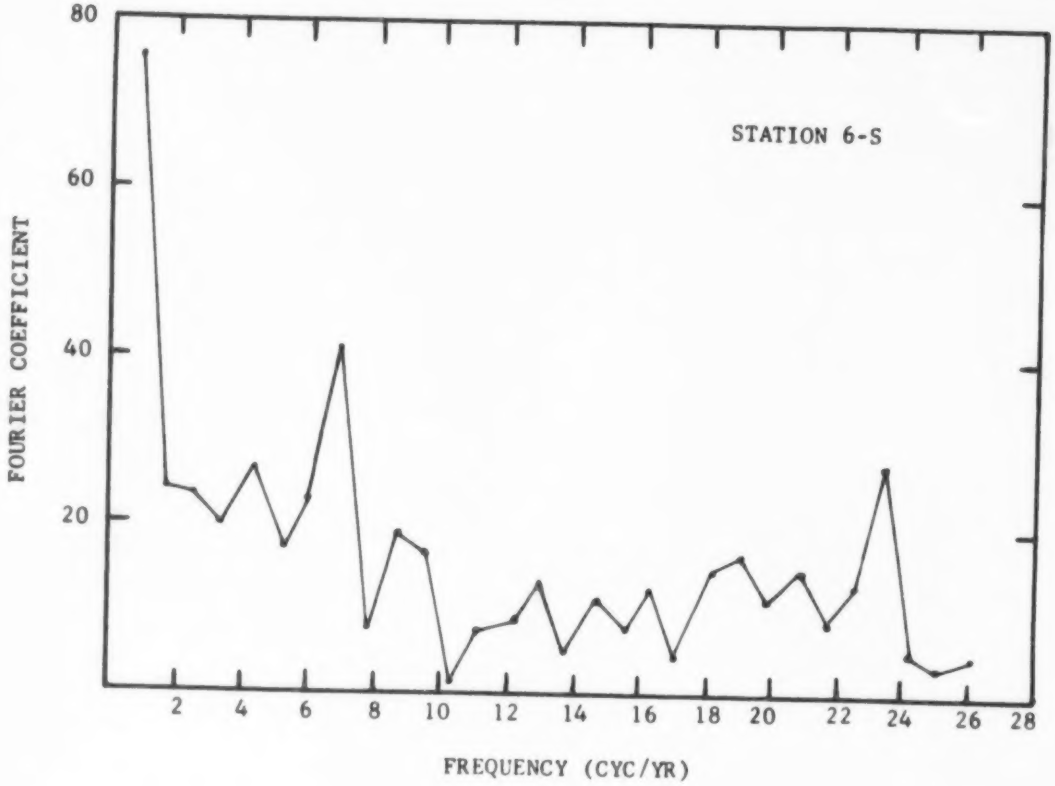


Figure 9

FOURIER TRANSFORM FOR ORTHO-PHOSPHATE

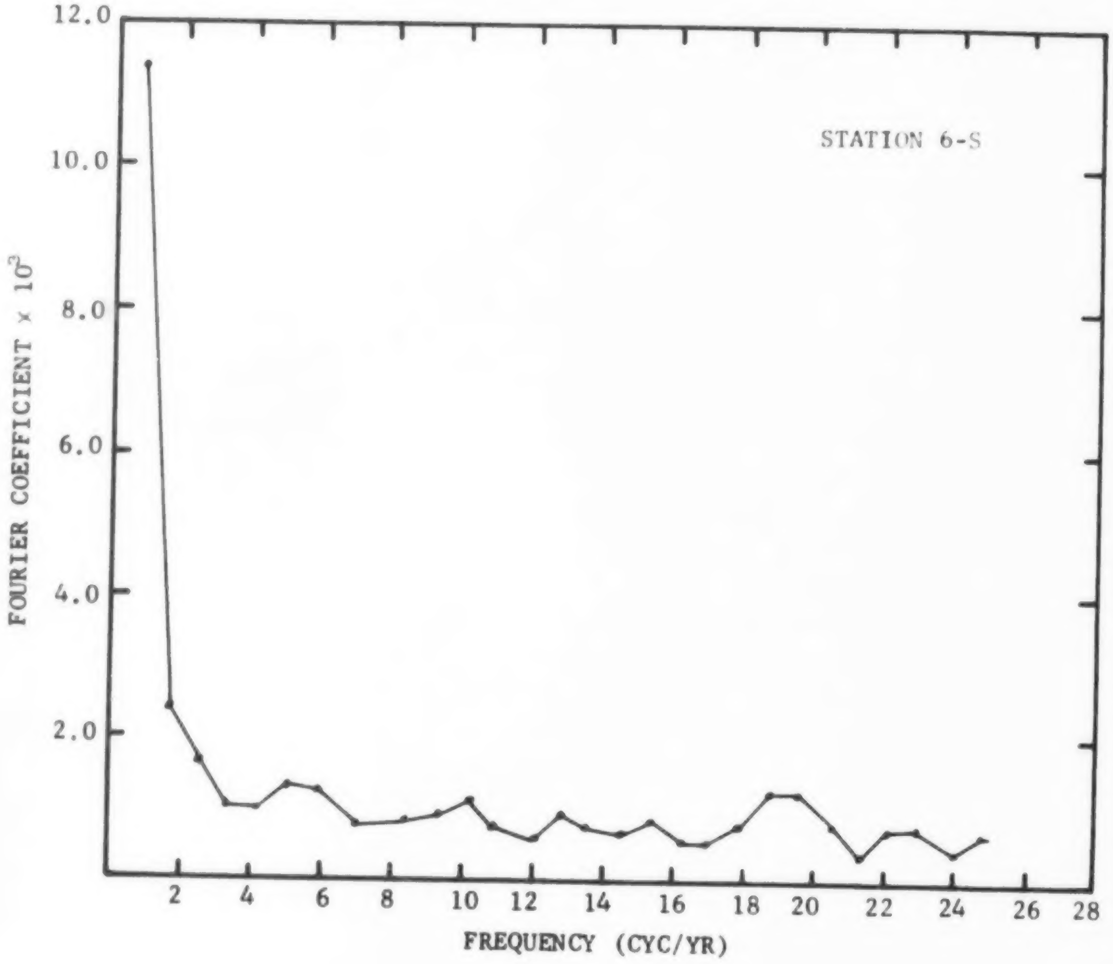


Figure 10

FOURIER TRANSFORM FOR NITRATE-NITROGEN

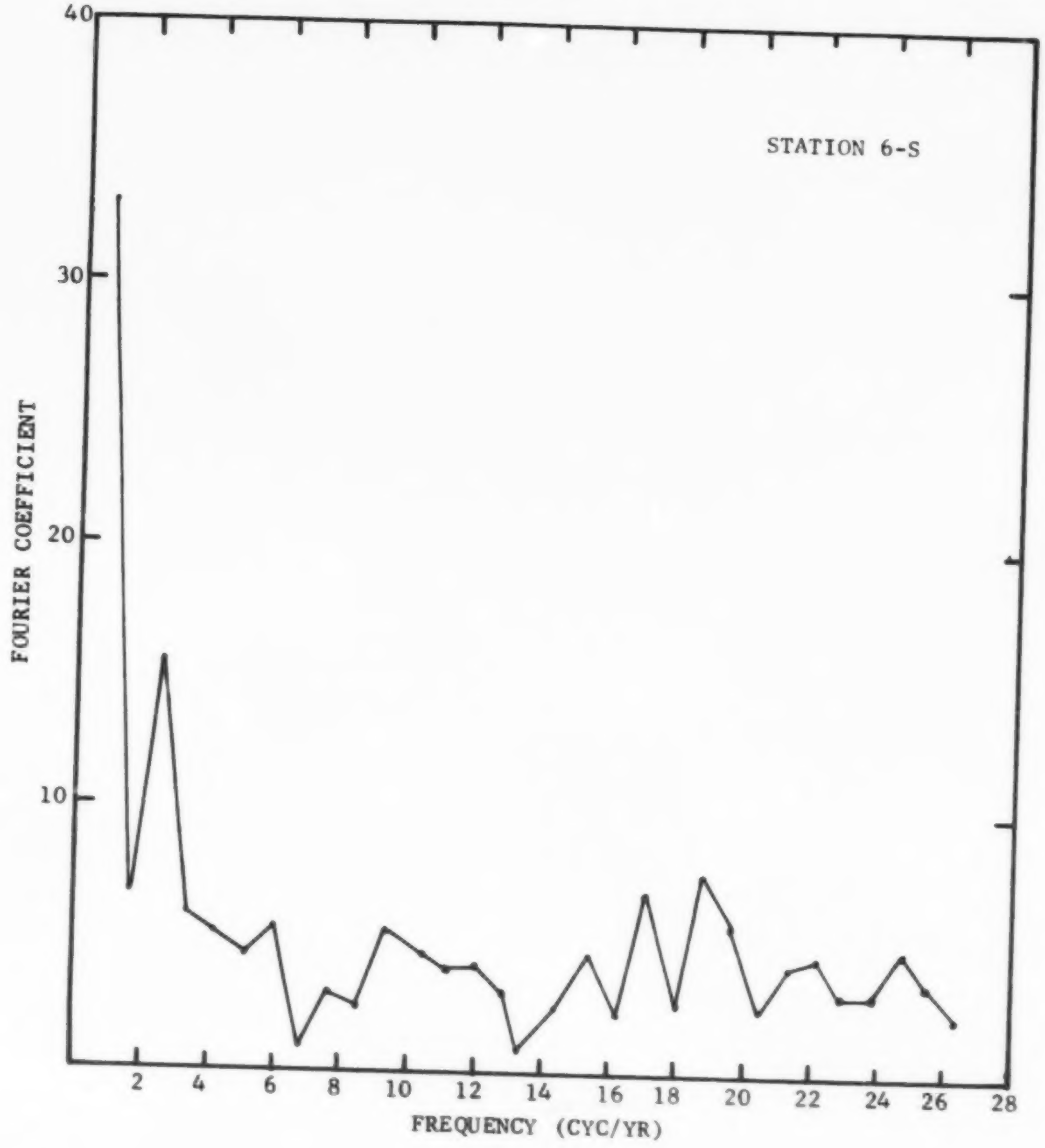


Figure 11

FOURIER TRANSFORM FOR DISSOLVED OXYGEN

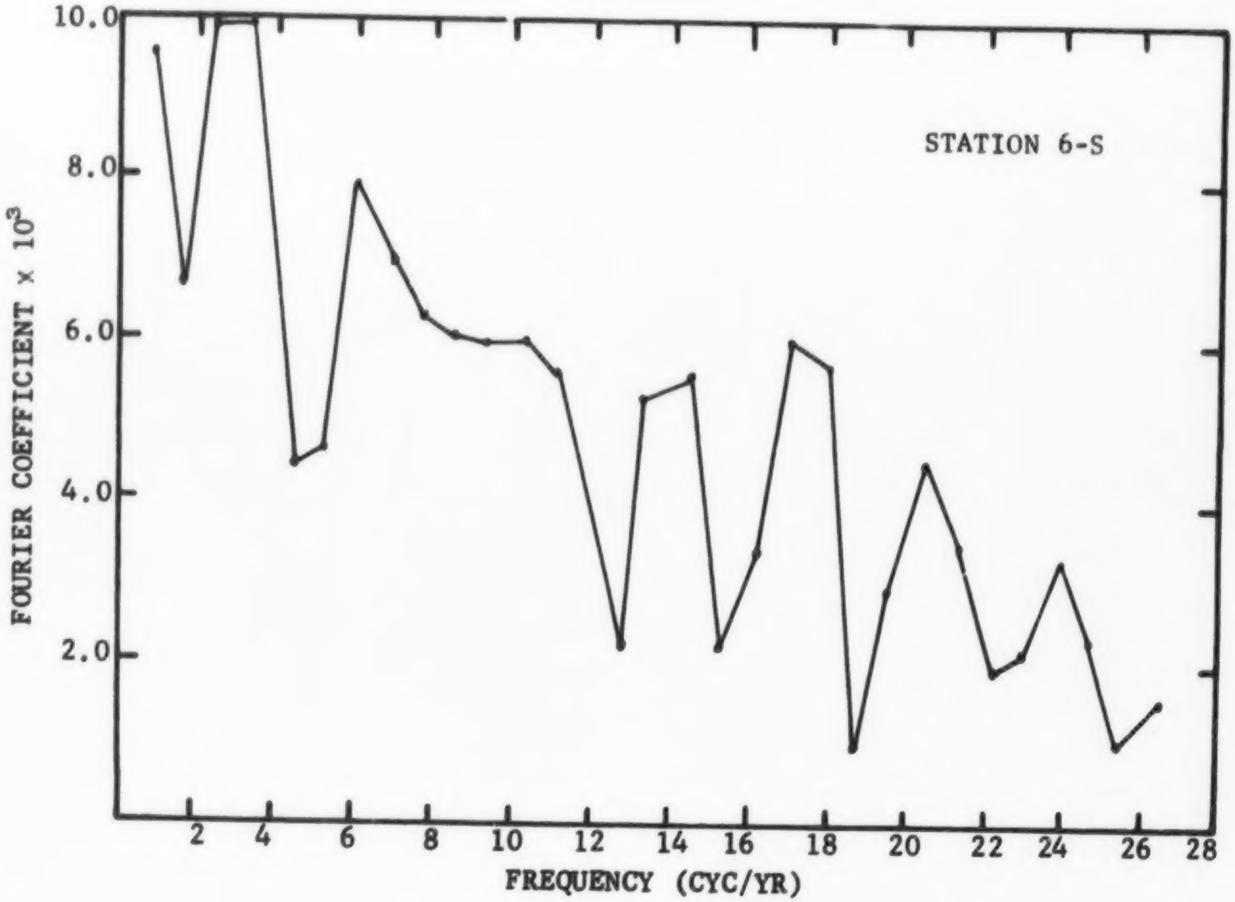


Figure 12

FOURIER TRANSFORM FOR SEDIMENT BACTERIA

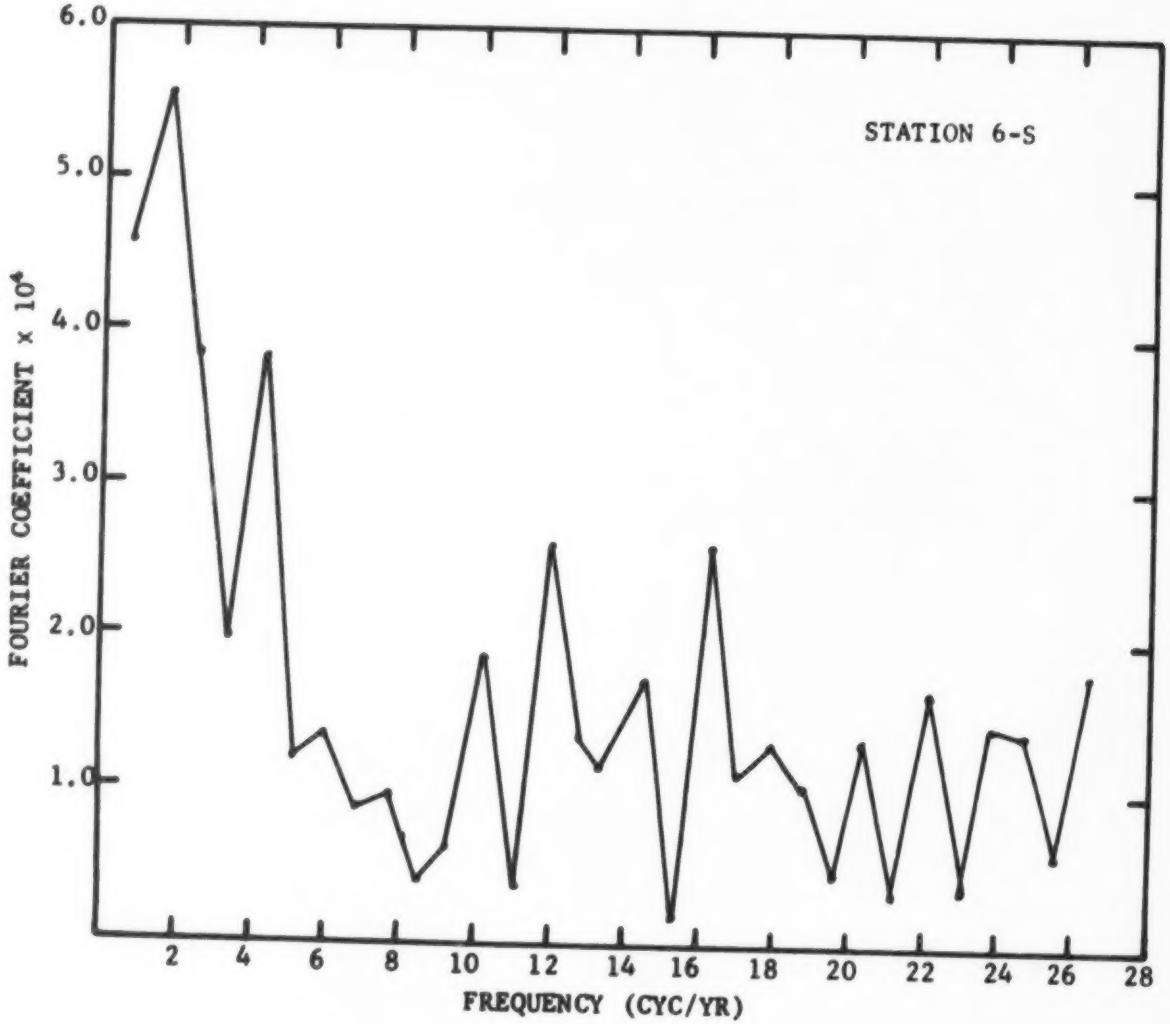


Figure 13

FOURIER TRANSFORM FOR WATER BACTERIA

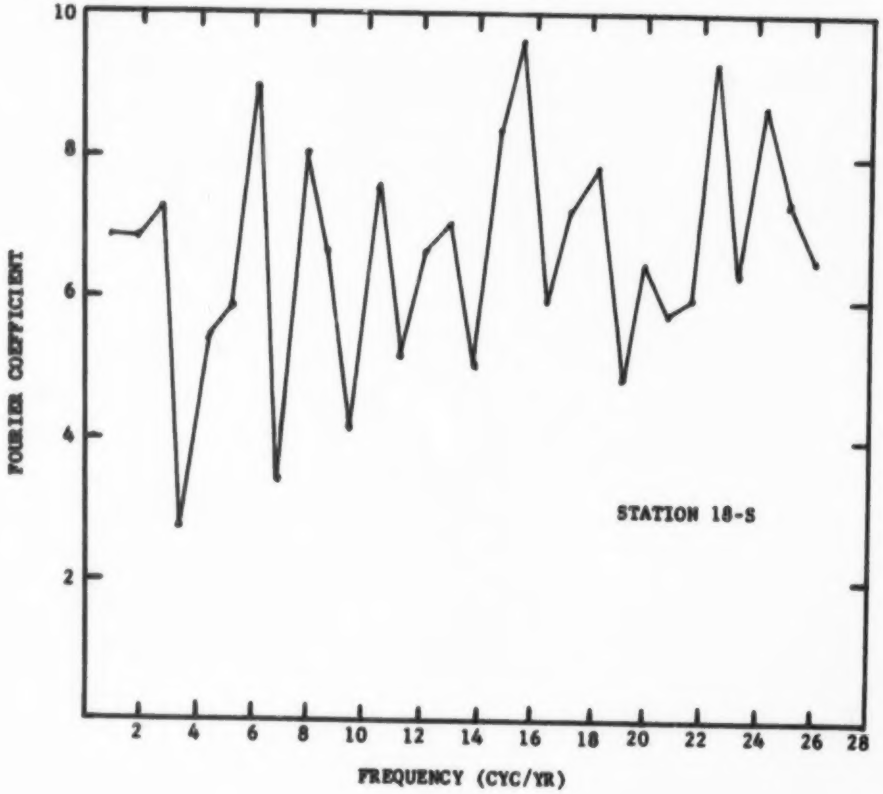


Figure 14

FOURIER TRANSFORM FOR pH

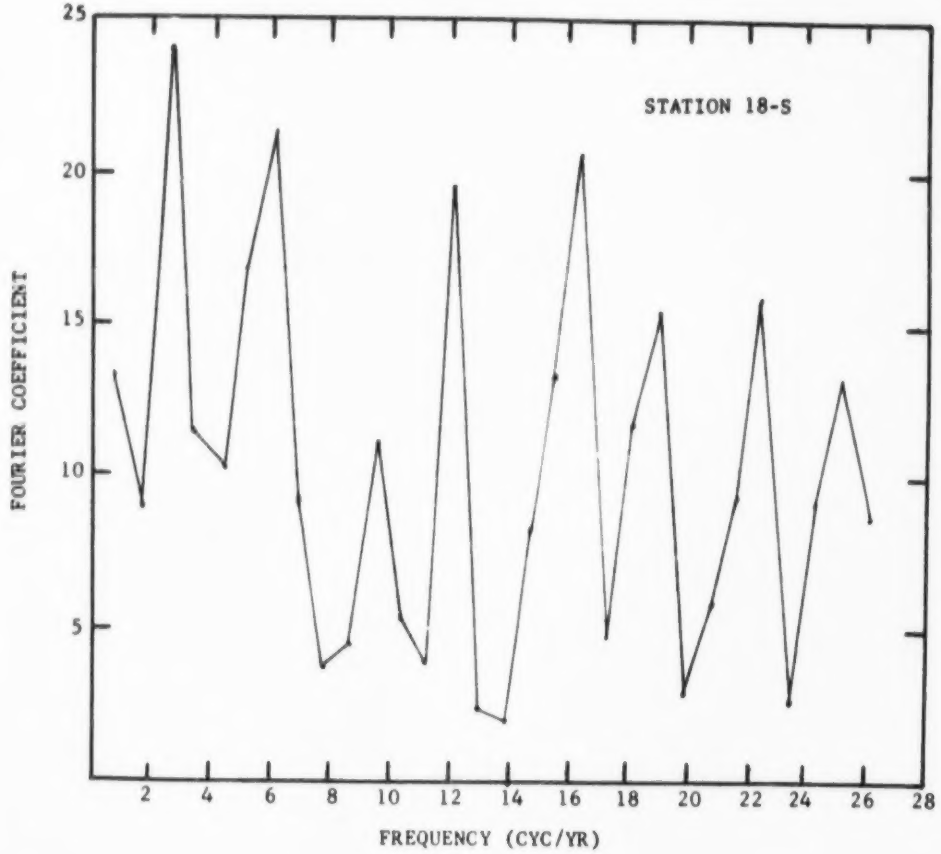


Figure 15

FOURIER TRANSFORM FOR TOTAL ORGANIC CARBON

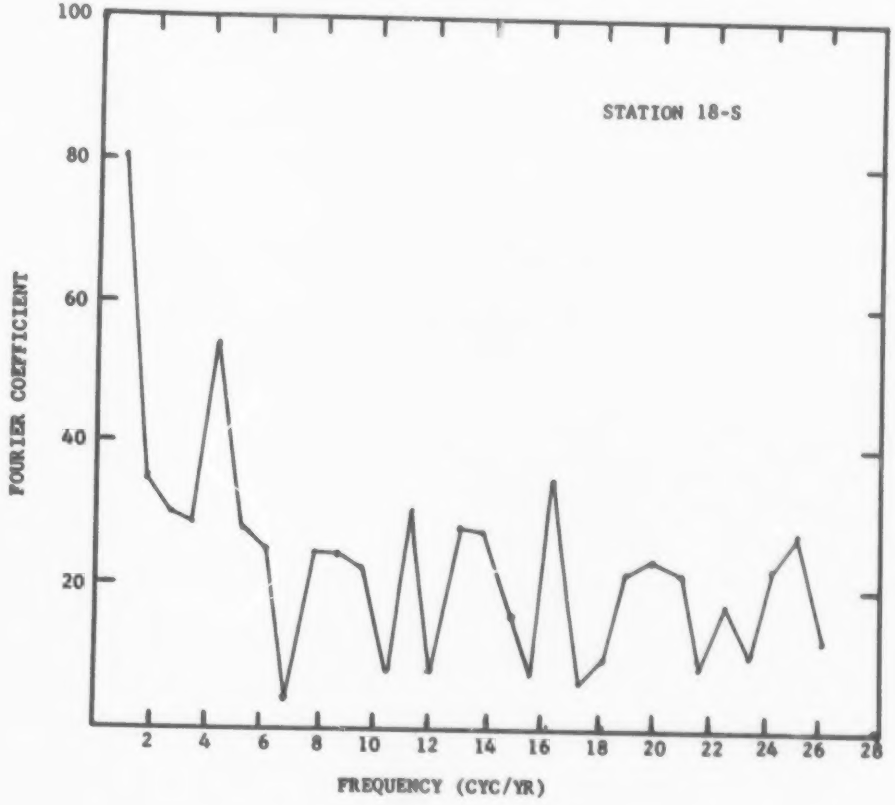


Figure 16

FOURIER TRANSFORM FOR TOTAL INORGANIC CARBON

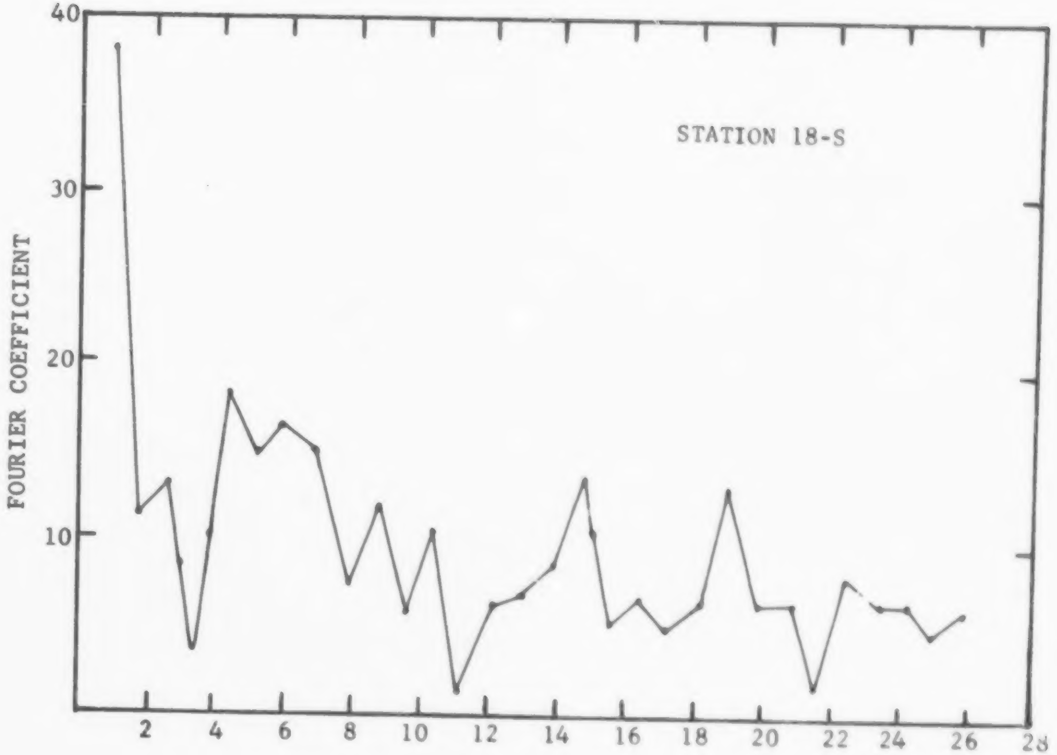


Figure 17

FOURIER TRANSFORM FOR CARBON DIOXIDE

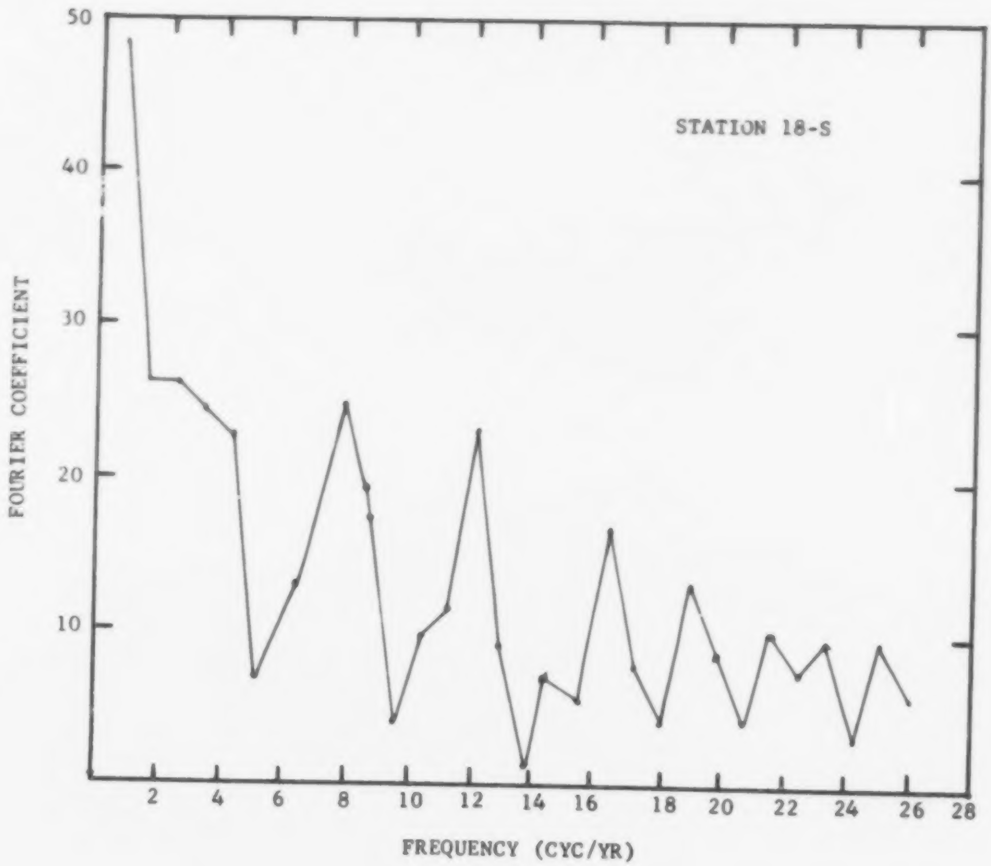


Figure 18

FOURIER TRANSFORM FOR BICARBONATE

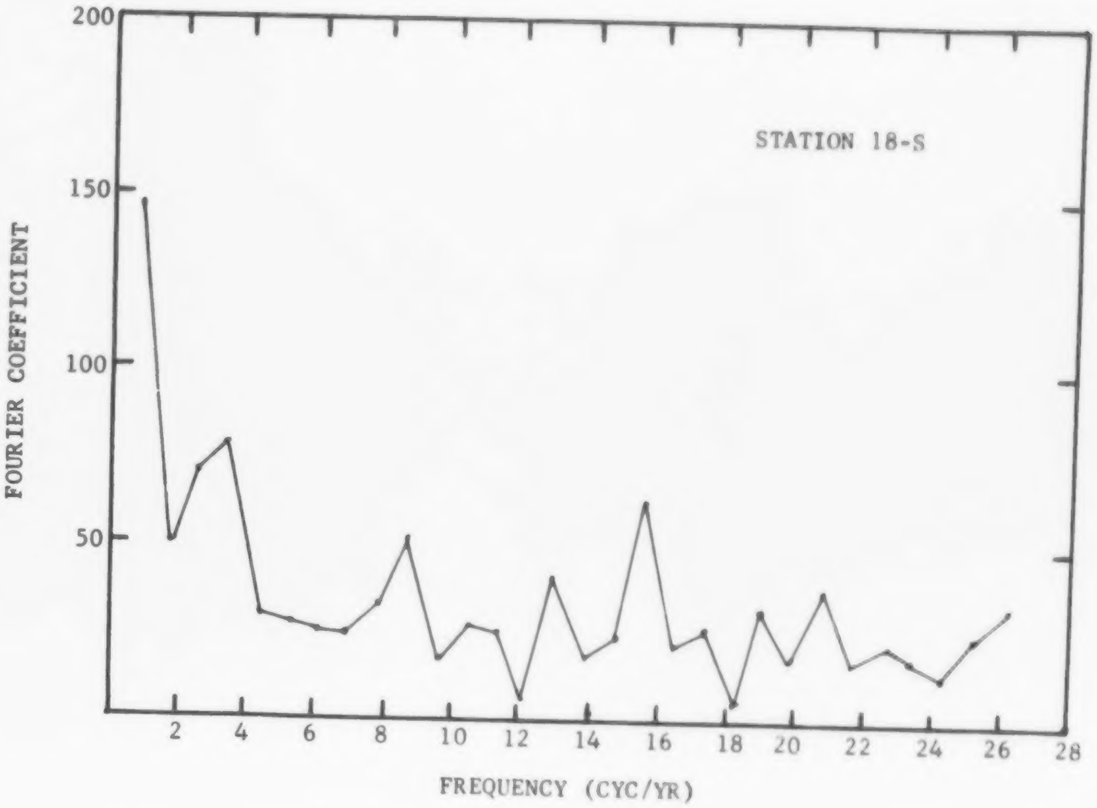


Figure 19

FOURIER TRANSFORM FOR TOTAL PHOSPHORUS

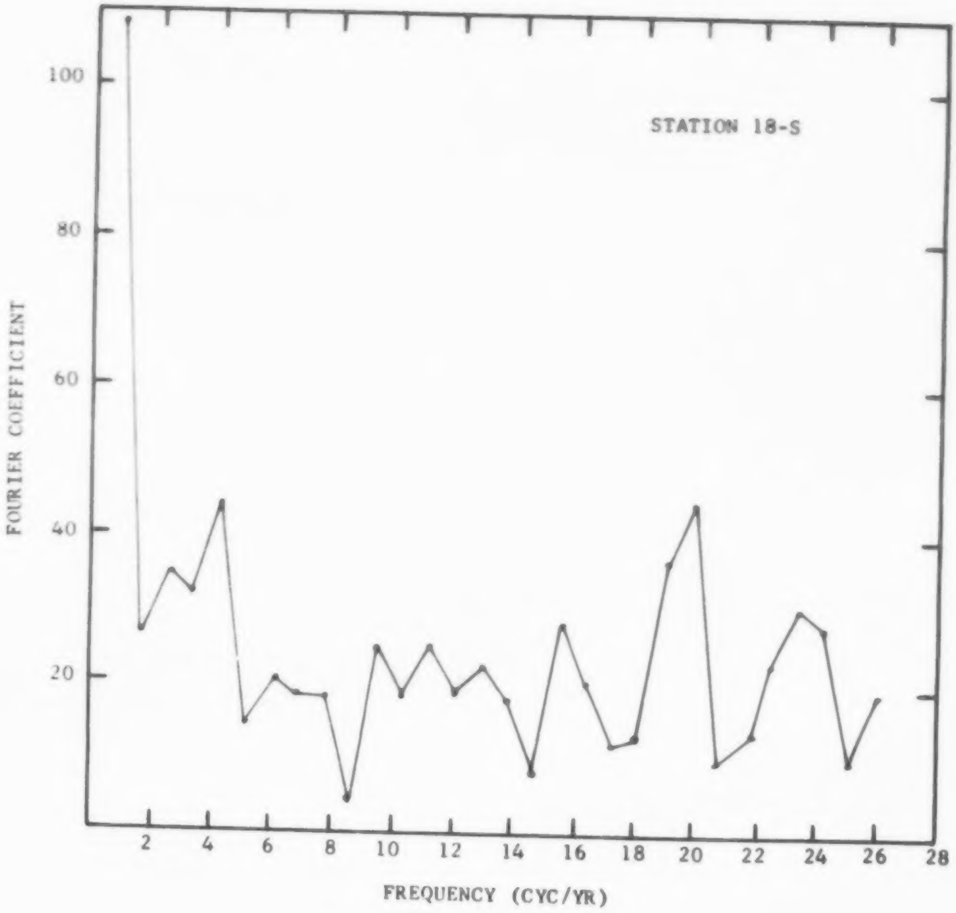


Figure 20

FOURIER TRANSFORM FOR ORTHO-PHOSPHATE

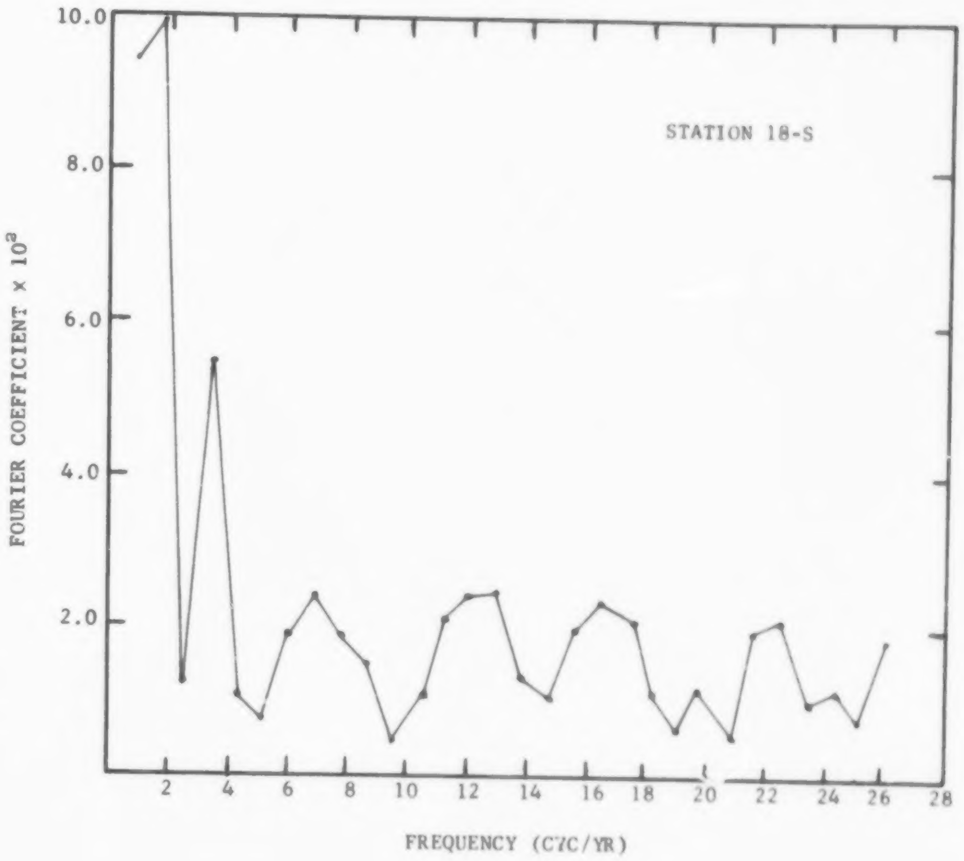


Figure 21

FOURIER TRANSFORM FOR NITRATE-NITROGEN

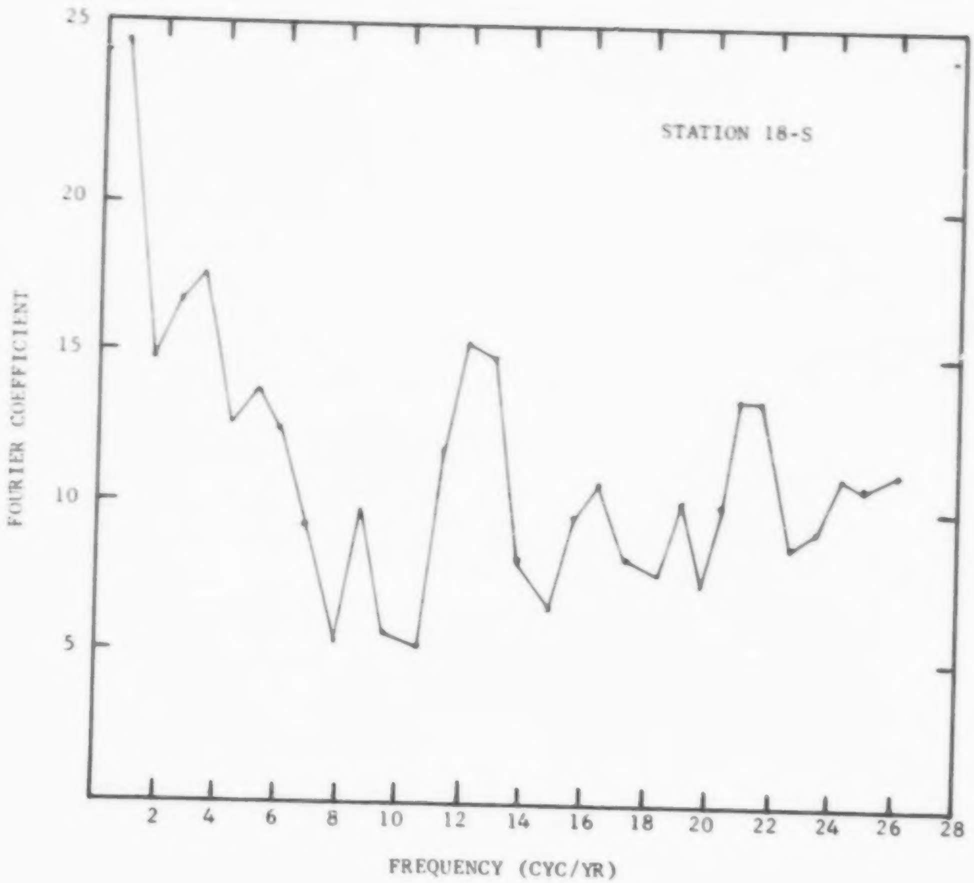


Figure 22

FOURIER TRANSFORM FOR DISSOLVED OXYGEN

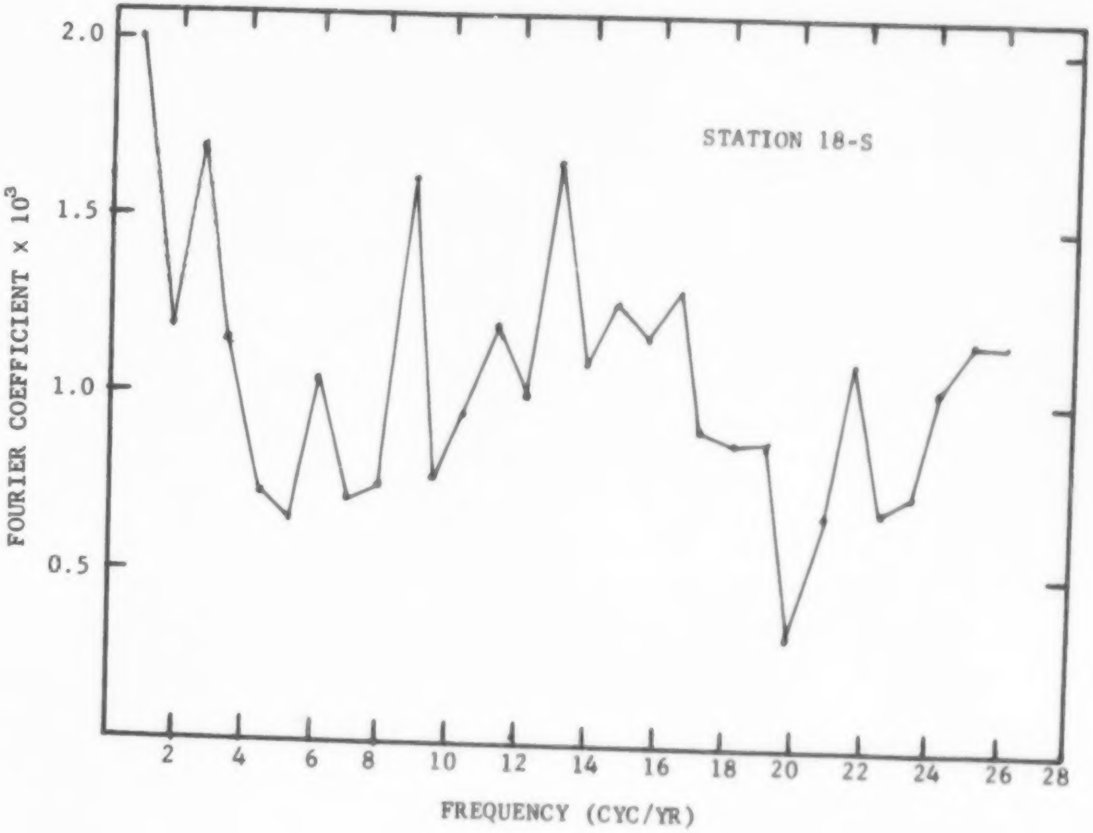


Figure 23

FOURIER TRANSFORM FOR SEDIMENT BACTERIA

Table 2. MAJOR OSCILLATORY FREQUENCIES OF MONITORED CONSTITUENTS AT STATION 6-S

Constituent	Frequency (cyc/yr)								
	1	2-3	5-6	8-10	11-13	14-17	19-22	24-25	26
pH	X	X	X	X	X				
TOC		X	X	X	X	X	X	X	
TIC	X	X		X	X	X	X	X	X
CO_2	X	X	X	X	X	X			
HCO_3^-	X	X	X	X		?	?	?	
TP	X	X	X		X	X	X	X	
$PO_4^{\equiv}$	X	X	X						
NO_3^-	X								
DO	X	X							
Sed. Bact.	X	X	X	X	X	X	X	X	
H_2O Bact.		X	X	X	X	X	X	X	

Table 3. MAJOR OSCILLATORY FREQUENCIES OF MONITORED CONSTITUENTS AT STATION 18-S

Constituent	Frequency (cyc/yr)								
	1	2-4	5-7	8-10	11-13	14-17	18-20	21-22	24-25
pH	X	X	X	X	X	X		X	X
TOC	X	X	X	X	X	X	X	X	X
TIC	X		X	X	X	X	X	X	X
CO_2	X	X	X	X		X			
HCO_3^-	X	X	X			X	X		
TP	X	X		X	X				
$PO_4^{\equiv}$	X	X					X		X
NO_3^-	X	X							
DO	X	X	X	X	X			X	
Sed. Bact.	X	X	X	X	X	X		X	X
H_2O Bact.									

Water bacteria, sediment bacteria, and all forms of inorganic carbon appear to cycle more rapidly in the perturbed stream (6-S) than in the stream surrounded by the climax forest (18-S). The inverse is true for nitrate-nitrogen, dissolved oxygen, and total organic carbon. These differences are very pronounced for nitrate-nitrogen and suspended and sediment bacteria. Based on preliminary data, total inorganic carbon, total organic carbon, total phosphorus, and bacterial concentrations are interacting parameters.

This preliminary study demonstrates the cyclic phenomena occurring in streams and illustrates the application of Fourier transforms in the analysis of such cyclic phenomena. Future work should involve the determination of phase angles of oscillation of major frequencies of nutrient cycles and the quantitative investigation of couplings between nutrient and biological cycles implied in this preliminary study.

SUMMARY

Data sets containing measurements of the concentrations of major nutrients and bacteria in two primary oligotrophic streams over a 64-week period were Fourier transformed. The transform coefficients as function of frequency indicate that non-random variations occurred in all measured parameters. Changes in nutrient and bacterial cycling patterns caused by clear-cutting of a watershed are illustrated by the differences between patterns in two streams, one in a climax forest and one in a clear-cut watershed.

ACKNOWLEDGMENTS

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REFERENCES

1. MacCrimmon, H. R. and J. R. M. Kelso. 1970. Seasonal Variation in Selected Nutrients of a River System. Fisheries Research Board of Canada 27:837-846.

2. Kelso, J. R. M. and H. R. MacCrimmon. 1969. Diel and Seasonal Variations in Physiochemical Limnology, Speed River, Ontario. Water Resources Research 5:1388-1394.

3. Wright, J. C. and I. K. Mills. 1967. Productivity Studies on the Madison River, Yellowstone National Park. Limnol. Oceanogr. 12:568-577.

4. Todd, R. L., Pat C. Kerr, W. A. Swank, D. L. Brockway, J. Douglas, and C. D. Monk. 1973. Chemoautotrophic Nitrification and Nitrate Losses in an Eastern Deciduous Forest Floor Ecosystem. Presented at the Annual Meeting of the American Society of Limnology and Oceanography. Salt Lake City, Utah, June 10-14.

5. Kerr, Pat C., D. L. Brockway, Doris F. Paris, J. T. Barnett, Jr., and Marilyn F. Cox. 1974. The Fate of Carbon, Nitrogen, and Phosphorus in Inland Surface Waters. U.S. Environmental Protection Agency. (In press)

6. Morse, P. M. and H. Feshback. 1953. Methods of Theoretical Physics, Vol. I. McGraw-Hill Publishing Co., New York. 19 p.

7. Kreyszig, E. 1965. Advanced Engineering Mathematics. John Wiley and Sons, Inc., New York. 48 p.

8. Cooley, J. W. and J. W. Tukey. 1967. An Algorithm for the Machine Calculation of Complex Fourier Series. Math. Comp. 19(90): 297-301.

THE EFFECT OF HIGHER TROPHIC LEVEL COMPONENTS IN AN AQUATIC ECOSYSTEM MODEL

Ray R. Lassiter

INTRODUCTION

Within-season patterns of phytoplankton blooms, grazer and predator population peaks, fluctuations in decomposer activity, and variations in material cycling are prominent features of aquatic ecosystem dynamics. The processes of translocation and transformation of materials are expected to occur differently in systems that differ in patterns of these blooms and peaks. To provide a framework for studying material transformations and translocations in varying aquatic ecosystems a mathematical model was constructed and used in simulations. Simulations were run representing situations with and without consumers. After observations have been made of the fluctuation patterns of relatively well-understood materials, i.e., major nutrients, the model will be used to assess the probable behavior of various pollutants in systems with different magnitudes of fluctuations in the component populations.

This paper describes the rationale basic to such a model and compares results from versions of the model with and without higher trophic levels. It also compares the present model with a precursory model[1]; in this context, the present model is referred to as the "enhanced model."

THE MULTIPLE SPECIES MODEL CONCEPT AND A PRECURSORY MODEL

Many mathematical models lump all organisms of a single general type, in the researcher's view, into one compartment, e.g., phytoplankton. The multiple species model, in contrast, represents individual types of organisms as individual compartments. Eggers and Male[2] stated that models with species differentiation are necessary for questions concerning competition, predation, selection, and adaptation to be considered.

A model was developed[1] in which six algal "species" were represented. Included also was a simple, dynamic, inorganic chemical equilibrium system plus a set of organic chemicals not directly involved in the equilibria among the inorganic chemicals. The specific growth rates and gaseous exchange rates used in the model were temperature dependent. The algae were dependent upon light, carbon, nitrogen, and phosphorus for growth. Because of differences in temperature optima and differences in uptake efficiency and growth rates, the individual species competed with varying degrees of success as the season progressed. The result (Figure 1) was a seasonal pattern of "blooms" reminiscent of a growing season observable in natural waters (Figure 2). Note an early spring peak of growth when most nutrients were present in inorganic form, and a later season competition for cycling nutrients as the nutrient levels and the temperature changed.

To find the effect of physiological type upon the pattern of phytoplankton production, species 1 was removed from the simulation. The results are shown in Figure 3, A and B. Figure 3B shows the simulated phytoplankton standing crop through time with species 1 absent, and Figure 3A is the corresponding portion of Figure 1 redrawn to the same scale as Figure 3B. Note the same general pattern of total phytoplankton biomass. Removing one species did not greatly alter the total phytoplanktonic biomass production early in the season even when the species removed accounted for nearly the entire bloom when included in the simulation. The property of the model giving rise to this behavior cannot exist outside a multiple species model. This is a necessary property of models to be used to investigate situations in which species respond differently to various pollutants.

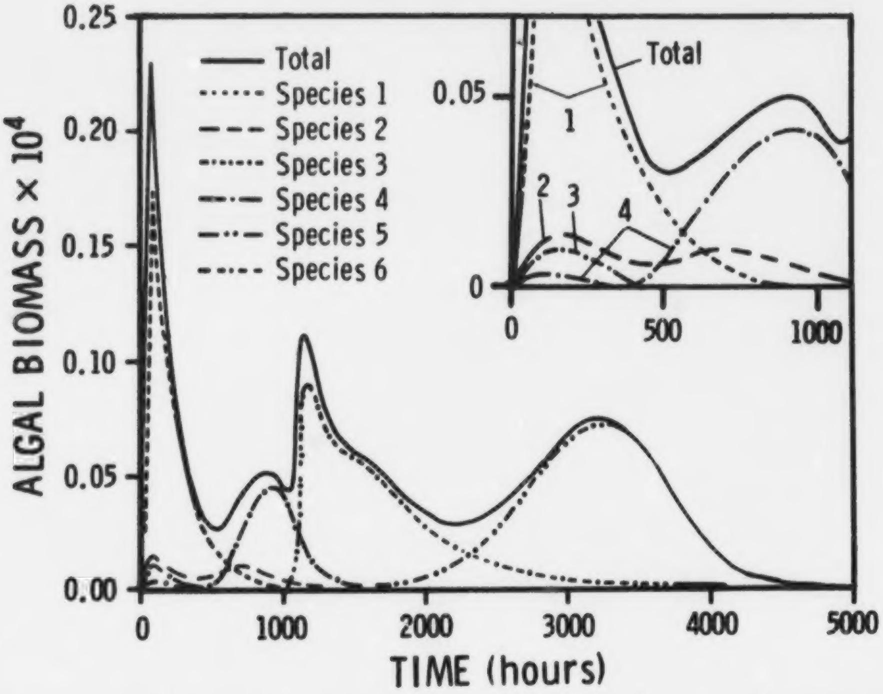

Figure 1. Simulated phytoplankton biomass through a growing season.

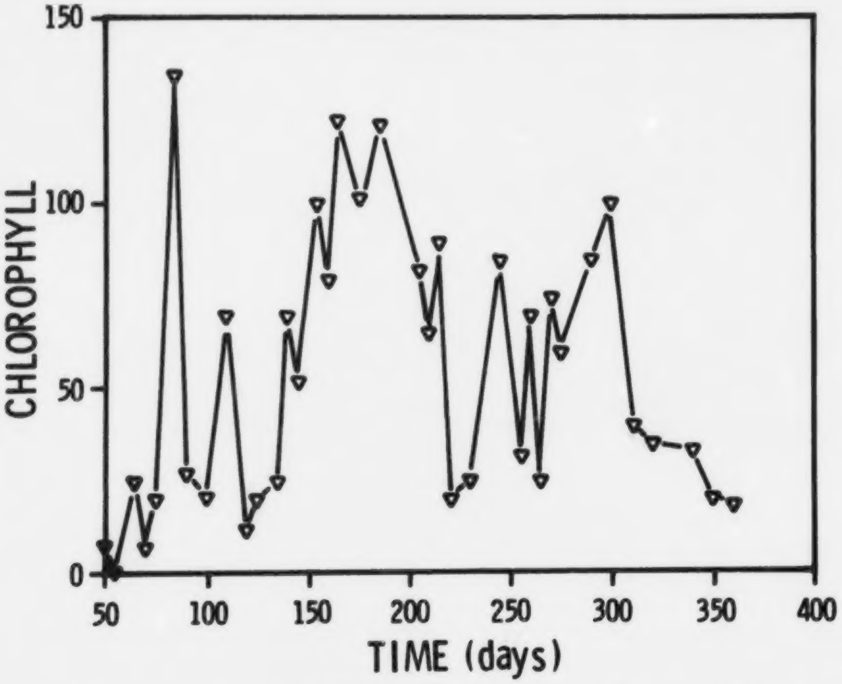

Figure 2. Pattern of chlorophyll during 1969 on a Potomac River sampling station. Data approximated from Figure 39 of O'Connor et al.[3]

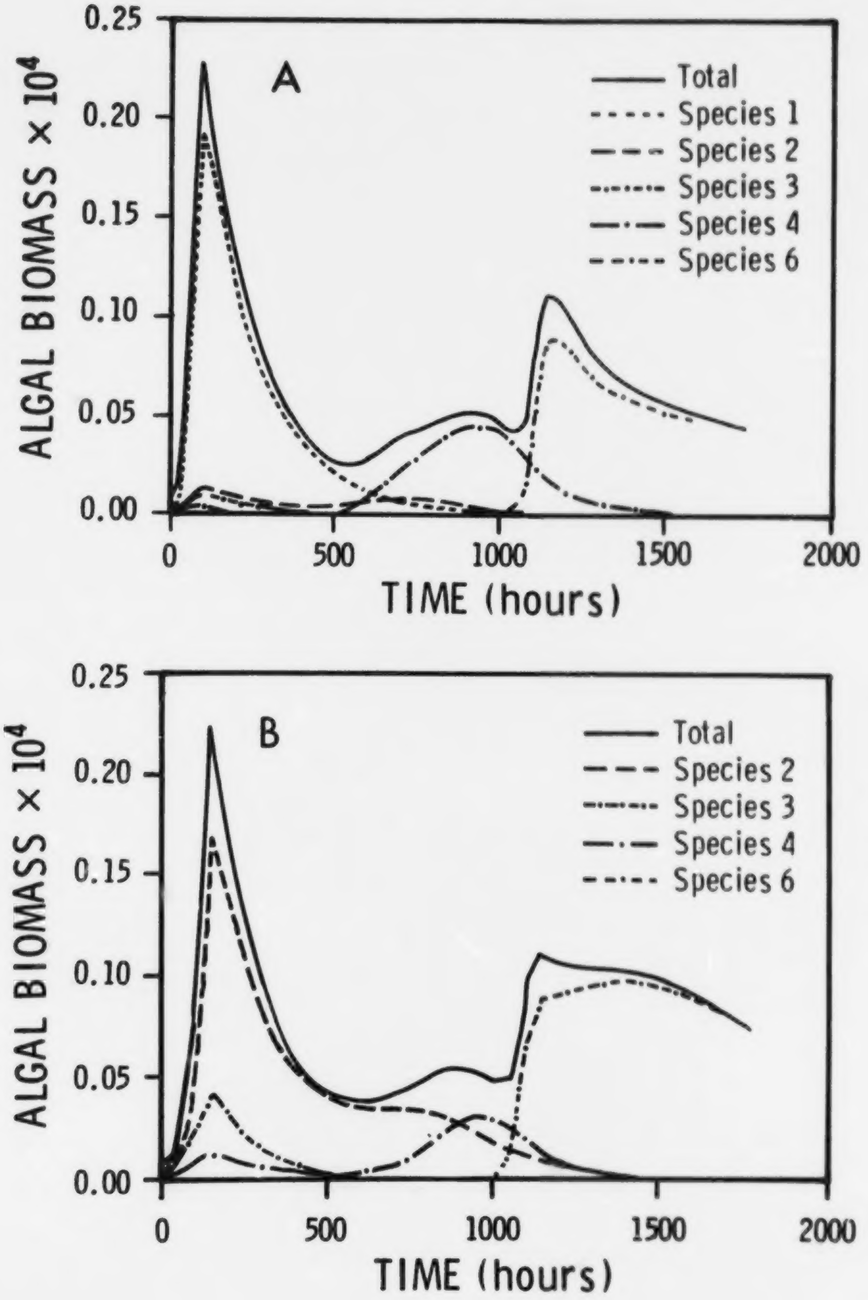

Figure 3. Simulated phytoplankton biomass over a portion of a growing season. Figure 3A is the same as the first part of Figure 1. Figure 3B shows a simulation with species 1, which accounted for virtually the entire initial bloom of Figure 3A, removed from the simulation.

THE ENHANCED MODEL

Many aspects of the enhanced model are identical to those of the precursory model. However, several differences and additions require some elaboration. These aspects include algal, bacterial, and zooplankton growth, a coupling structure comprised of the food web and other transfers and transformations, and the stoichiometric relations for the various transformations of materials. The structure is shown schematically in Figure 4. The main concepts are discussed below. The detailed system equations will be found in Appendix 1 and Appendix 2.

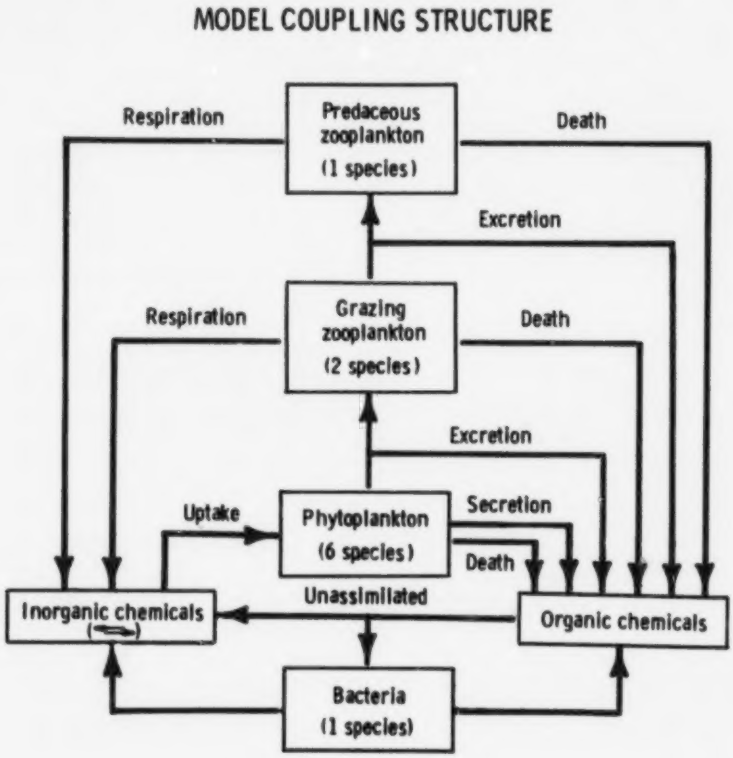
MODEL COUPLING STRUCTURE
Predaceous zooplankton (1 species)
Respiration
Death
Excretion
Grazing zooplankton (2 species)
Respiration
Death
Excretion
Phytoplankton (6 species)
Uptake
Secretion
Death
Unassimilated
Inorganic chemicals
Organic chemicals
Bacteria (1 species)

Figure 4. Schematic representation of the enhanced model.

Algal Growth

As in the precursory model, algal growth was made a function of light, temperature, and the concentration of CO_2, ammonia nitrogen, and mono- and dihydrogen phosphate ions in the water. The Michaelis-Menten model was used as the basis for the growth rate terms used in both cases. However, in the precursory model, growth rate was made a function of a product of three terms, each of the form $C/(K + C)$, in which C is the concentration of a major nutrient, and K is the concentration of the same nutrient that results in half maximal growth. Although frequently used, this form has several disadvantages[1]. In the enhanced model, growth rate is a function of the minimum of the set of terms $\{C_1/(K_1+C_1), C_2/(K_2+C_2), C_3/(K_3+C_3)\}$ where subscripts refer to specific nutrients (see Appendix 1). Using this construction, the growth rate constant as found by experimentation appears to be more nearly applicable to the multiple factor situation.

Bacterial Growth

Bacteria were not explicitly present in the precursory model. Instead, organic compounds were assumed to decay at rates proportional to their concentrations. In the enhanced model bacteria were included in order to transform organic carbon, nitrogen, and phosphorus into their respective inorganic forms. In general, bacteria utilize inorganic ions in addition to organic compounds; but for this model bacterial growth rate was not made an explicit function of inorganic ions. Any inorganic ions assimilated were assumed to be used immediately after they were released from the organic form. The growth rate of bacteria was made a function of the minimum of the set of terms $\{C_5/(K_5+C_5), C_6/(K_6+C_6), C_7/(K_7+C_7)\}$, i.e., a function of the concentrations of organic compounds only (for subscripts, see Appendix 1).

Zooplankton Growth

Zooplankton were not represented in the precursory model. Consequently no effect of grazing or other effect of higher trophic levels could be studied. In the enhanced model, a Michaelis-Menten expression is used to describe satiation, i.e., the reduced grazing rate per unit prey with increasing prey density. The specific growth rate (μ) term for predator i is

$$\mu_i = \hat{\mu}_i \frac{\sum_{j=1}^{m} p_{ij} B_j}{K_i + \sum_{j=1}^{m} p_{ij} B_j} \tag{1}$$

in which $\hat{\mu}$ is maximal growth rate,

p is a weighting constant defining the predation rate by the i^th predator on the j^th prey type relative to the rate on any other prey type,

B is biomass of prey,

K is the concentration of prey as modified by the weight, p, that results in half maximal predator growth, and

m is the number of prey types.

If the predator feeds by random encounter of the prey individuals, regardless of species, the p_{ij} are all equal.

The Coupling Structure of the Model

The dynamic part of the model is a set of highly coupled differential equations, which in turn are coupled to a set of algebraic equations for chemical equilibrium. The equations are coupled in that terms for disappearance of a material (living or non-living) in one equation have corresponding terms for appearance in one or more other equations. For example, the disappearance of dissolved inorganic material may be accompanied by an appearance of algae, and the disappearance of algae may be accompanied by appearance of zooplankton and organic material. Disappearances are negative terms and appearances positive terms in the differential equations.

All couplings are shown in the appendices. Many of the terms involved in the couplings contain stoichiometric coefficients, n_{ij}, in which i indicates a source (reactant) and j indicates a product. These will be discussed in the following section. Examples of the couplings are discussed below.

If $\underline{G_a}$ is the vector of growth rates of algae, all yields are 100% efficient ($\underline{Y} = 1$), and $\underline{n_{2a}}$ is the vector of stoichiometric coefficients relating moles of CO_2 to moles to algae, then the loss rate of CO_2 due to algal growth is $-\underline{n_{2a}G_a}$. If the vector of respiration rates of heterotrophic organisms is $-\underline{R_z}$, then the corresponding rate of change for dissolved CO_2 is $\underline{n_{h2}R_z}$ and for dissolved O_2 is $-\underline{n_{1h}}\ \underline{R_z}$. If the vector of excretion loss rates of organic carbon from zooplankton is $\underline{E_z}$, then rate of increase of organic carbon is $\underline{n_{5h}}\ \underline{E_z}$. (For further definition of the symbols, see Appendix 1.)

The food web relationships are represented by growth rates, predation rates that sustain those growth rates, and other transformations occurring as a result of predation. Of the total growth rate of species i, (μ_i, Equation 1), the fraction F_{ki}, given by

$$F_{ki} = \frac{p_{ik}\ B_k}{\sum_{j=1}^{m} p_{ij} B_j} \tag{2}$$

is attributable to predation upon species k.

Accounting for the yield (Y_i), the biomass of predators able to take prey (B_i), and the stoichiometric coefficient relating a mole of prey to a mole of predator (n'_{ki}), the predation rate on an individual of species k is given by

$$P_k = \sum_{i=1}^{r} n'_{ki}\ B_i \left(\frac{\mu_i}{Y_i}\right) \left(\frac{p_{ik}\ B_k}{\sum_{j=1}^{m} p_{ij}\ B_j}\right) \left(\frac{\sum_{j=1}^{m} p_{ij}\ B_j}{K_i + \sum_{j=1}^{m} p_{ij}\ B_j}\right)$$

$$= B_k \sum_{i=1}^{r} n'_{ki}\ B_i \frac{\mu_i\ p_{ik}}{Y_i\ (K_i + \sum_{j=1}^{m} p_{ij}\ B_j)} \tag{3a}$$

Alternatively, since the growth rate of the ith predator is

$$G_i = \mu_i B_i \frac{\sum_{j=1}^{m} P_{ij} B_j}{K_i + \sum_{j=1}^{m} P_{ij} B_j} ,$$

substitution into equation 3a results in

$$P_k = B_k \sum_{i=1}^{r} \frac{n'_{ki} P_{ik} G_i}{Y_i \sum_{j=1}^{m} P_{ij} B_j} , \qquad (3b)$$

or using Equation (2)

$$P_k = \sum_{i=1}^{r} \left(\frac{n'_{ki}}{Y_i}\right) F_{ki} G_i . \qquad (3c)$$

The transfer of unassimilated algae to the organic pool and the stoichiometry of the transformation are discussed in the following section along with other aspects of stoichiometry.

When the system is completely coupled, and when no net losses or gains to the system are included, there should be conservation of mass. To check on proper system coupling and stoichiometry in computer models, one might leave off net system gains and losses until conservation of mass is achieved, then add export and import rates to simulate open system dynamics.

Stoichiometric Computations

One aspect of ecosystem modeling that is often neglected is the stoichiometric relationships that must be maintained in transformation of elements from one form to another. Cordeiro et al.[4] and Verhoff and Smith[5] have simulated systems in which complete cycles were modeled and stoichiometry was considered. This aspect of system modeling is especially important when material cycling is studied. Thus the following method of computing the stoichiometric coefficients was developed for the enhanced model.

Because a unit of consumed material may differ in atomic ratios of the principal elements from a unit of consumer protoplasm, assimilation of the consumed material is incomplete. That is, some of the consumed material must necessarily be released by the consumer in order to maintain its atomic ratios. This situation also sets the minimum rate of consumption per unit increase in consumer. The actual rate is increased by a factor for inefficiency of assimilation. This factor may equal one as is assumed in this model for assimilation by algae, but in general it will be greater than one.

To illustrate the computation of a stoichiometric coefficient, let algal composition be $C_{100}\ N_{15}\ P_1$ and zooplankton composition be $C_{80}\ N_{16}\ P_1$. The ratios of numbers of atoms in a mole of zooplankton to those in a mole of algae form the set:

$$\left\{\frac{80}{100}\ ,\ \frac{16}{15}\ ,\ \frac{1}{1}\right\}$$

The largest member of this set is the stoichiometric coefficient representing the minimum number of moles of algae required to supply a one-mole increase of zooplankton:

$$n'_{az} = \max\left\{\frac{80}{100}\ ,\ \frac{16}{15}\ ,\ \frac{1}{1}\right\} = \frac{16}{15} ,$$

or stated in another way, the reciprocal of the largest member (15/16) is the number of moles theoretically available for consumer biomass production per mole phytoplankton consumed. The quantity actually required is greater than the minimum calculated above because of assimilative inefficiencies. If the assimilative efficiency is y_{za} (moles consumer biomass produced per mole theoretically capable of being produced), the stoichiometric coefficient for this transfer is

$$n^*_{az} = \frac{1}{y_{za}}$$

and the overall coefficient

$$n_{az} = n'_{az}\ n^*_{az} \tag{4}$$

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$$P_k = B_k \sum_{i=1}^{r} \frac{n'_{ki} \, p_{ik} \, G_i}{Y_i \sum_{j=1}^{m} p_{ij} B_j} , \qquad (3b)$$

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The rate of consumption of an individual of phytoplankton species k, P_k, may now be written more compactly. Defining the terms $F_{ki}\ G_i$ from Equation (3c) as A_{ki}, in which $i = 1, 2, \ldots, r$, and r is the number of predators, the equation becomes

$$P_k = \underline{n_{ki}}\ \underline{A_{ki}} \qquad (5)$$

in which $\underline{n_{ki}}$ is the vector of stoichiometric coefficients for transfer of the k^{th} prey to the i^{th} predator, and

$\underline{A_{ki}}$ is the vector of weighted prey biomass values, as weighted by the vulnerability of the k^{th} prey to the i^{th} predator.

These computations relate moles of algae to moles of zooplankton. However, since the elemental composition of algae is assumed to be different from that of zooplankton, some of the material must be transformed to substances other than zooplankton. For example, carbon, at 100 atoms per mole of phytoplankton and 80 atoms per mole of zooplankton, must be liberated by zooplankton at the rate of 20 atoms per mole of algae eaten simply to maintain the integrity of the zooplankton protoplasmic composition. To illustrate, assume that the liberated carbon becomes organic carbon. A stoichiometric coefficient is needed for this transfer. The stoichiometric coefficient may be expressed as

$$n_{a^{p}_{z}5} = \frac{\text{moles of organic carbon produced}}{\text{moles of predator formed}} \qquad (6)$$

in which $n_{a^{p}_{z}5}$ is the stoichiometric coefficient relating the appearance of organic carbon to the formation of zooplankton biomass from the assimilation of phytoplankton.

This coefficient may be used with the growth rate of the predator to compute the rate of production of organic carbon. Other coefficients may be computed to use with other variables, each coefficient being the partial derivative of organic carbon with respect to the variable of interest. A coefficient with the units specified above may be obtained as follows:

$$n_{a_z^{p_5}} = \frac{x_1 n_{az} - x_2}{x_3} \tag{7}$$

in which x_1 is the number of carbon atoms per mole of phytoplankton,

n_{az} is the number of moles of phytoplankton taken per mole of predator produced, the stoichiometric coefficient as defined in Equation (4),

x_2 is the number of carbon atoms per mole of predator, and

x_3 is the number of carbon atoms per mole of organic carbon.

The numerator, $x_1 n_{az} - x_2$, represents the number of carbon atoms produced as organic carbon per mole of predator formed. Referring to the definitions of x_1, x_2, and x_3, the units for the stoichiometric coefficient may be determined by

$$U(n_{a_z^{p_5}}) = \frac{\left(\frac{a}{m_1}\right)\left(\frac{m_1}{m_2}\right) - \left(\frac{a}{m_2}\right)}{\frac{a}{m_3}}$$

in which $U(n_{a_z^{p_5}})$ is read "the units of the term $(n_{a_z^{p_5}})$,"

a is the number of carbon atoms,

m_1 is moles of phytoplankton,

m_2 is moles of zooplankton,

m_3 is moles of organic carbon.

When simplified the units m_3/m_2 match those specified in Equation (6).

Equation 7 may be applied as well to any by-product material as to organic carbon. If used as a general formula,

x_1 is number of atoms of any element per mole of some consumed material,

n_{az} is number of moles of the material that are consumed per mole of consumer produced,

x_2 is number of atoms of the element per mole of consumer,

x_3 is number of atoms of the element per mole of the by-product,

and for generality,

$n_{a_2^1 3}$ replaces $n_{a_z^p 5}$, the subscript $a_2^1 3$ signifying that the transfer occurs because organism 2 eats organism (or material) 1 with material 3 appearing as a by-product.

Where two or more by-products (e.g., both organic carbon and CO_2) result, this formulation becomes more involved but no different in principle.

The rate of appearance of organic carbon is

$$E_z = n_{a_z^p 5} G_z$$

and, in general, for the rate of appearance of material x from predator 2 feeding on prey 1

$$E_x = n_{a_2^1 x} G_2$$

This equation states that for each mole of prey eaten or predator formed, $n_{a_2^1 x}$ moles of by-product are produced.

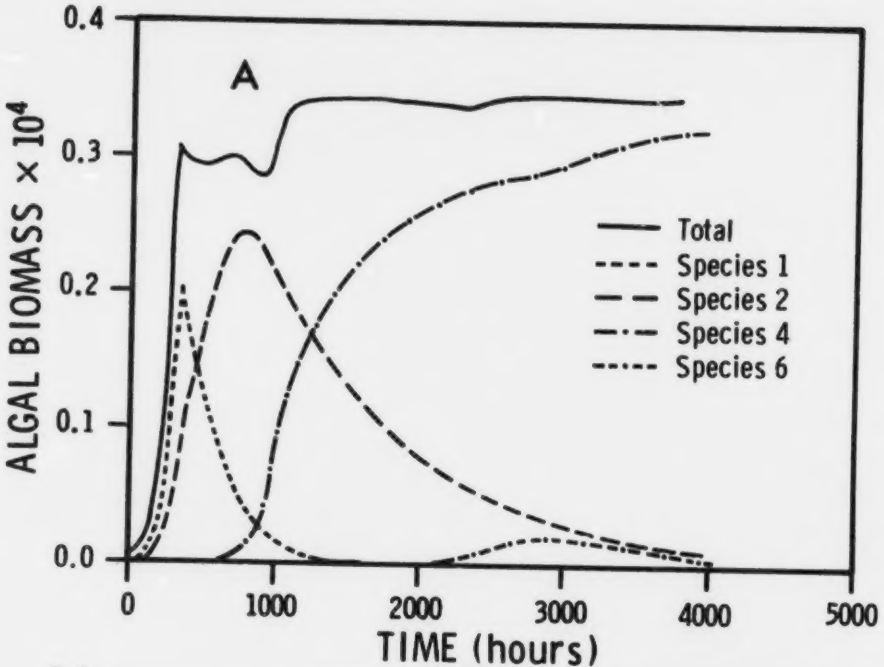
The computation of these coefficients remains applicable for at least two additional model refinements. First, in the enhanced model each species in a given trophic level is assumed to be of the same elemental composition and no organism is assumed to feed on more than one trophic level. Therefore any consumer eats food all of the same elemental composition. This is clearly a gross oversimplification. The method of computing the stoichiometric coefficients, n_{ij}, will remain applicable for a model not restricted in this manner. Second, in this and most models, the atomic ratios of the principal elements in the organisms are assumed to remain constant over time. However, the compositions of single-celled organisms are known to be, to some degree, a function of the concentration of nutrients in the external medium. Metazoans change body composition with age and with varying accumulation of carbohydrates and fats as energy storage. Future models might include such factors, and the above method of computing stoichiometric coefficients will apply to these model refinements as well.

The Effect of Higher Trophic Levels

The role of consumers has often been overlooked or minimized in aquatic ecosystem models. To investigate this aspect of system functioning, trial computer runs were made with and without consumers included in the model and with all other factors the same. Figure 5A shows the phytoplankton biomass from a run without consumers. The enhanced model without the consumers is similar to the precursory model, yet the results are very different (compare Figure 1 with Figure 5A). These differences arise partly from differences in the growth models, as previously described, but mostly from the relatively low natural death rates for phytoplankton used in the enhanced model. The low phytoplankton death rates were used to allow the effects of death rates from zooplankton grazing to show up more distinctly.

The results obtained using the model with consumers are strikingly different from those obtained without consumers. Figure 5B shows the phytoplankton biomass from a run with consumers. The two periods of rapid reductions in biomass resulted from zooplankton grazing. Figure 6 shows total phytoplankton biomass and biomass of the two grazer populations as simulated by the model. The two periods of decline in phytoplankton correspond to the periods of maximum rate of increase of the zooplankton. Zooplankton declined because they reduced their food supply and because temperature became unfavorable.

The initially obvious differences in standing crop of algae are not the only differences observable when zooplankton are added to the model. Differences in nutrient cycling can be noted for any nutrient. For example Figure 7A shows the concentration of total inorganic carbon. The dips in the curve correspond to periods of rapid phytoplankton growth (hence inorganic carbon uptake). The



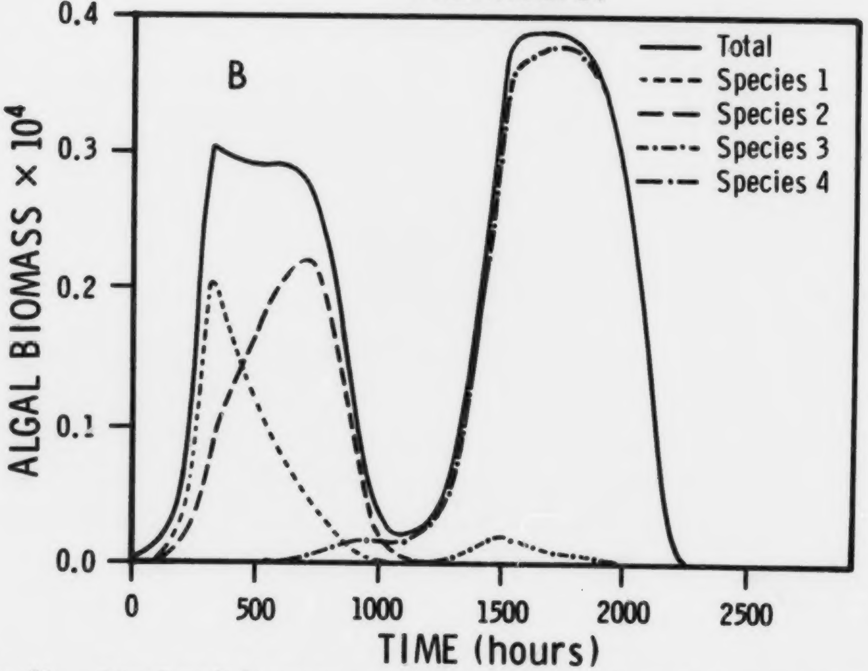


Figure 5A. Simulated biomass of phytoplankton through a growing season for a biological community consisting of six phytoplankton species and bacteria.

Figure 5B. Simulated biomass of phytoplankton through a growing season for a biological community consisting of two species of primary consumers and one species of secondary consumer in addition to the species in the simulation of Figure 5A.

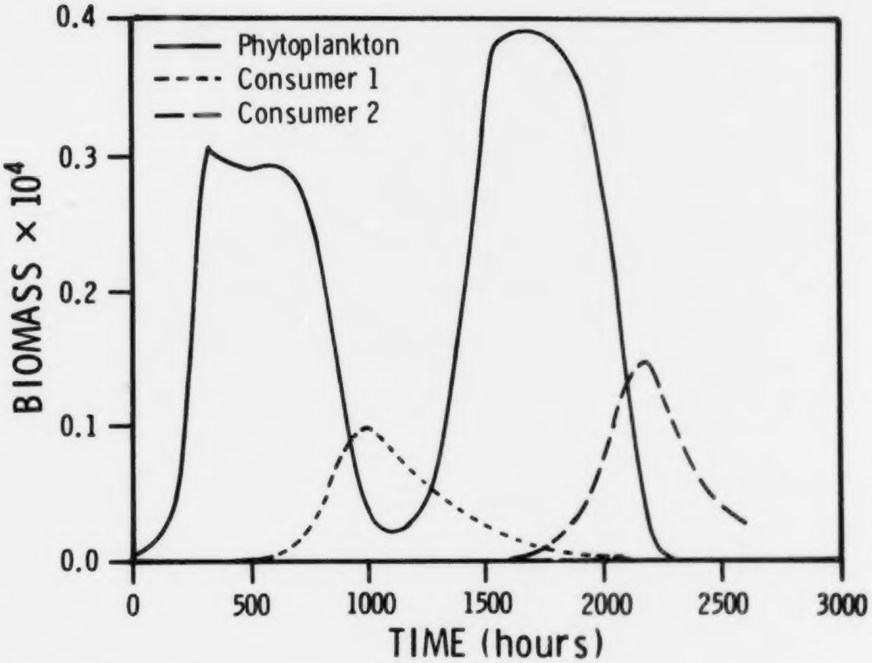
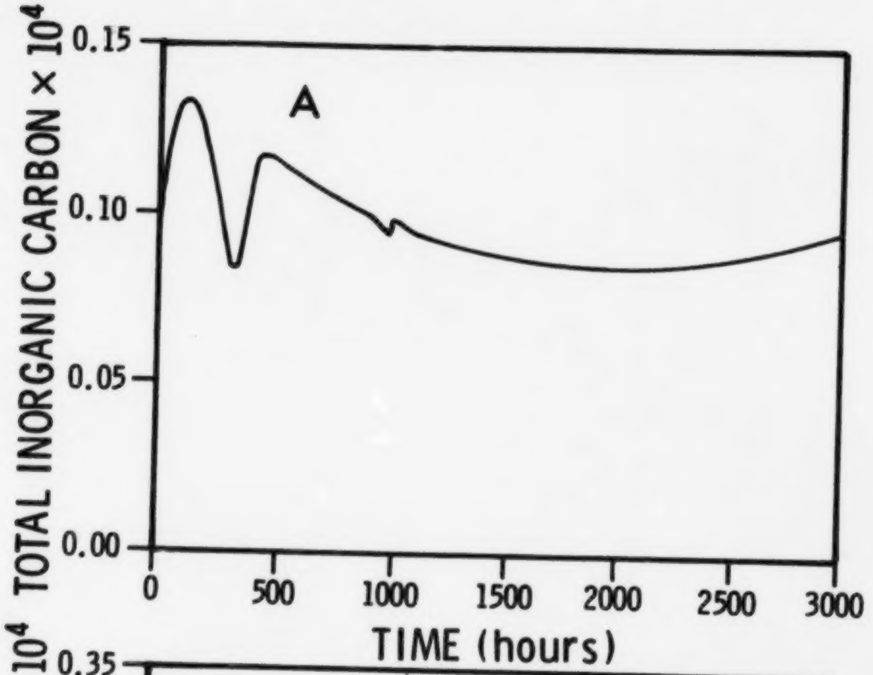


Figure 6. Total phytoplankton biomass and the biomass of each of two species of primary consumer. Note the correspondence of maximum rate of increase of consumer corresponding to maximum rate of decrease of phytoplankton.

general curved appearance in the graph reflects the varying solubility of CO_2 as temperature varies. A comparison with Figure 7B shows the greater irregularity associated with the situation in which zooplankton participate in nutrient cycling.

Another manifestation of the role of consumers is the bacterial dynamics. Figures 8, A and B, show the bacterial standing crop without and with consumers, respectively. Without consumers an initial peak was followed by stabilization at a relatively low level. The reason for this stabilization was limitation of bacteria by nitrogen. When consumers were added, nutrients were made available at higher rates and increased amounts as evidenced by several growth periods and a higher maximum standing crop. However, there was greater variation in the production of organic



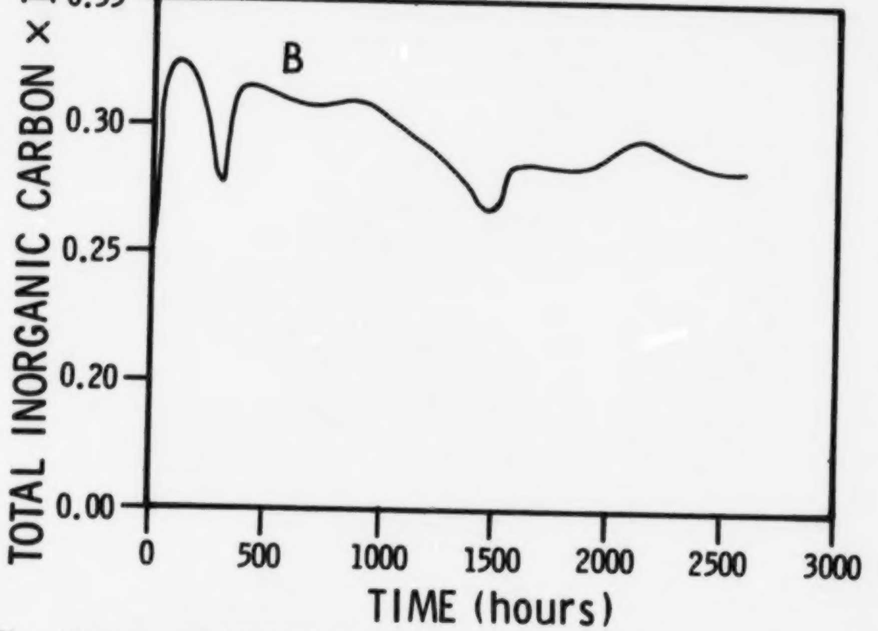
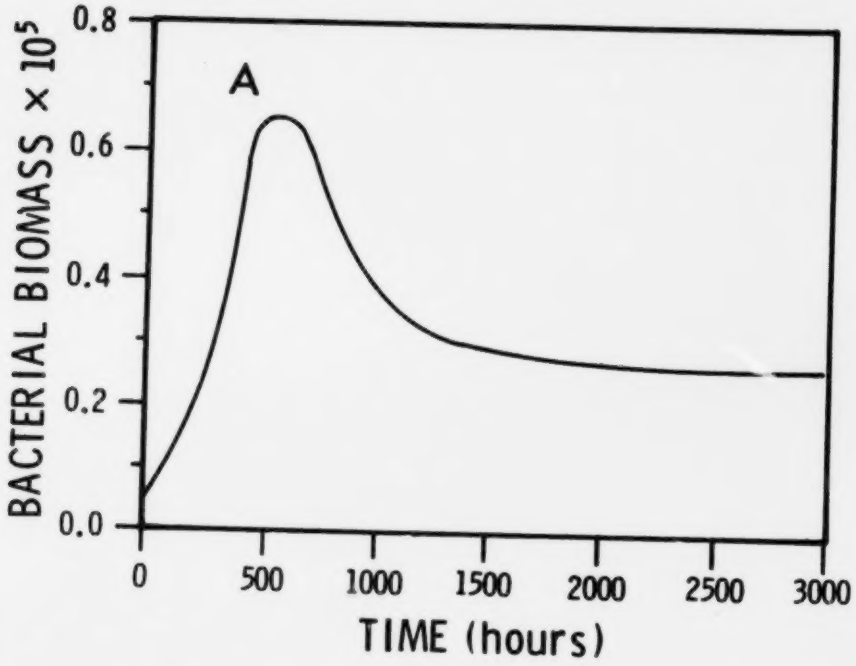


Figure 7A. Pattern of total inorganic carbon over part of a growing season for a simulation with a biological community consisting of six phytoplankton species and one bacterial species. Dips correspond to period of maximal phytoplankton growth.

Figure 7B. Pattern of total inorganic carbon over part of a growing season for a simulation with a biological community including two species of primary consumer and one species of secondary consumer in addition to the community for Figure 7A.



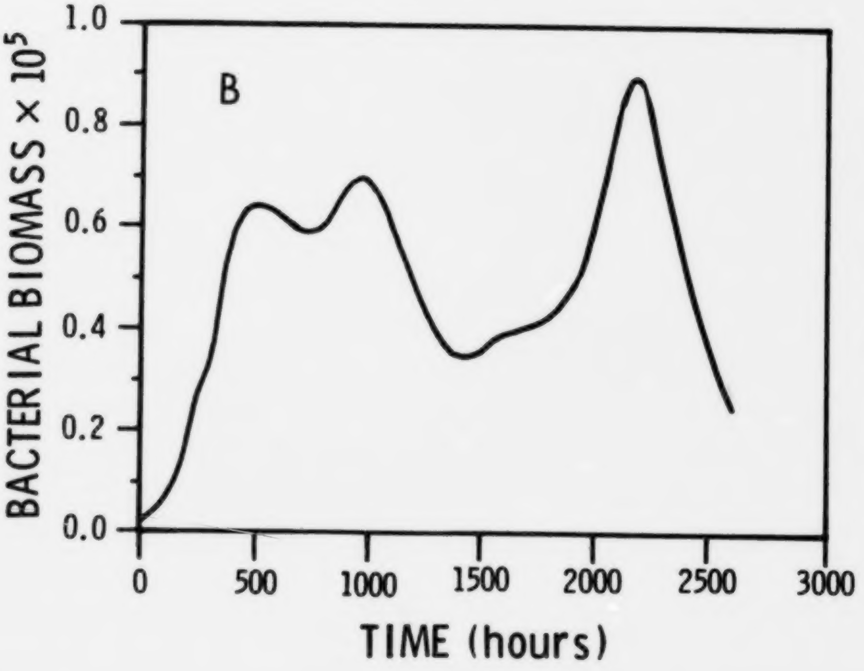


Figure 8A. Simulation of pattern of bacterial biomass for biological community without consumers.

Figure 8B. Simulation of pattern of bacterial biomass for biological community with consumers.

material throughout the growing season, as evidenced by fluctuations in bacterial standing crop. Also the bacterial population declined near the end of the season because the consumers virtually eliminated the phytoplankton populations, thus reducing the rate of organic material production. The reduction in bacterial population further resulted in a low rate of transformation of aquatic materials to inorganic nutrients for phytoplankton growth.

The specific growth patterns predicted by experimental computer simulations cannot be expected to correspond directly to any natural situation. However, the changes in growth patterns brought about by including consumers in the simulation illustrate the importance of higher trophic levels in ecosystem modeling. In addition to their direct effect on prey populations, consumers play a significant role in nutrient cycling and therefore in setting the patterns and levels of abundance of all types of aquatic organisms.

Models such as the one described are useful in studying the effects of various nutrient regimes on biomass production and growth rate limitation. Biomass limitation occurred in all simulations. The only limit to biomass production was nutrient availability; therefore a maximum biomass was reached when utilization reduced nutrients to concentrations at which population loss rates equalled or exceeded uptake rates. Zooplankton consumers, by recycling nutrients, may slightly increase the upper limit for phytoplankton (Figure 5, A and B) and bacteria (Figure 8, A and B) but on the other hand they can ultimately reduce the standing crop (Figure 6).

CONCLUSIONS

Dynamic models considering many factors, e.g., a food web, stoichiometric relations, and conservation of mass, can be built and used effectively to investigate aquatic ecosystem functioning.

This model indicates that consumers are important in the dynamics of every system component examined.

Such models have potential in studying many phenomena associated with aquatic ecosystems and the effects of pollution in these systems. The effect of removing species or otherwise altering such systems may be considered. Such models may be used to plan large scale experiments or studies with ecosystems. They can aid in determining the types and quantities of measurements needed and can provide a framework for data analysis.

REFERENCES

1. Lassiter, R. R. and D. K. Kearns. Phytoplankton Population Changes and Nutrient Fluctuations in a Simple Aquatic Ecosystem Model. In: Modeling the Eutrophication Process, Proceedings of a Workshop Held at Utah State University, Logan, Utah, September 5-7, 1973 (E. J. Middlebrooks, Donna H. Falkenborg, and T. E. Maloney, eds.). Utah State University and U. S. Environmental Protection Agency. Logan, Utah. PRWG136-1. Utah Water Research Laboratory, College of Engineering, Utah State University. November 1973. p. 131-138.

2. Eggers, D. M. and L. M. Male. The Modeling Process Relating to Questions about Coniferous Lake Ecosystems. In: Proceedings - Research on Coniferous Forest Ecosystems - A Symposium (J. F. Franklin, L. J. Dempster, and R. H. Waring, eds.). Washington, U. S. Government Printing Office, 1972. #0101-0233. p. 33-36.

3. O'Connor, D. J., R. V. Thomann, and D. M. Di Toro. Dynamic Water Quality Forecasting and Management. U. S. Environmental Protection Agency, Washington, D. C. EPA-660/3-73-009. 170 p.

4. Cordeiro, C. F., W. F. Echelberger, and F. H. Verhoff. Rates of Carbon, Oxygen, Nitrogen, and Phosphorus Cycling Through Microbial Populations in Stratified Lakes. In: Modeling the Eutrophication Process, Proceedings of a Workshop Held at Utah State University, Logan, Utah, September 5-7, 1973 (E. J. Middlebrooks, Donna H. Falkenborg, and T. E. Maloney, eds.). Utah State University and U. S. Environmental Protection Agency. Logan, Utah. PRWG136-1. Utah Water Research Laboratory, College of Engineering, Utah State University. November 1973. p. 111-120.

5. Verhoff, F. H. and F. E. Smith. Theoretical Analysis of a Conserved Nutrient Ecosystem. J. Theoret. Biol. 33:131-147, 1971.

APPENDIX 1

THE SYSTEM OF DYNAMIC EQUATIONS

The equations of the model are summarized below to indicate the couplings. For explanation of the terms refer to Appendix 2.

Dissolved Oxygen (C_1)

$$\frac{dC_1}{dt} = k_1(C_{1s} - C_1) + n_{1a}(G_a + S) - n_{1h}(R_h + E_{75})$$

Dissolved CO_2 (C_2)

$$\frac{dC_2}{dt} = k_2(C_{2s} - C_2) - n_{2a}(G_a + S) + n_{2h}(R_h + E_{75})$$

Dissolved Ammonia (C_3)

$$\frac{dC_3}{dt} = -n_{3a}G_a + n_{3h}(R_h + E_{76})$$

Dissolved Phosphate (C_4)

$$\frac{dC_4}{dt} = -n_{4a}G_a + n_{4h}(R_h + E_{76})$$

Total Organic Carbon (C_5)

$$\frac{dC_5}{dt} = n_{5a}(D_a + S) + n_{5h}D_h + n_{a_z p_5}E_z - n_{5b}G_b$$

Total Organic Nitrogen (C_6)

$$\frac{dC_6}{dt} = n_{6a}D_a + n_{6h}D_h + n_{a_z p_6}E_z - n_{6b}G_b$$

Total Organic Phosphorus (C_7)

$$\frac{dC_7}{dt} = n_{7a}D_a + n_{7h}D_h + n_{a_z p_7}E_z - n_{7b}G_b$$

Phytoplankton (B_i, $i=1, 2, \ldots, 6$)

$$\frac{dB_i}{dt} = G_i - D_i - P_iB_i$$

Bacteria (B_7)

$$\frac{dB_7}{dt} = G_7 - D_7 - R_7$$

Herbivorous Zooplankton (B_8, B_9)

$$\frac{dB_j}{dt} = G_j - D_j - P_jB_j - R_j$$

Carnivorous Zooplankton (B_{10})

$$\frac{dB_{10}}{dt} = G_{10} - D_{10} - R_{10}$$

APPENDIX 2

EXPLANATIONS OF TERMS USED IN APPENDIX 1

The terms comprising the equations summarized in Appendix 1 are presented below in a somewhat expanded form. Some deviation from the notation employed in the text will be noted. For example where the text uses $\underline{G_a}$ as a vector of growth rates, G_a is used below as a sum of growth rates. The whole terms $\underline{n_{xa}G_a}$ (text) and $n_{xa}G_a$ (Appendix 1) are the same where $\underline{n_{xa}}$ is a constant vector, the case for the actual equations used and presented in Appendix 1.

C_{1s} is the molar concentration of dissolved oxygen in water when the water is saturated with oxygen.

C_{2s} is the molar concentration of dissolved CO_2 in water when the water is saturated with CO_2.

D_a is the sum of non-predatory death rates of all phytoplankton species. It is assumed to be proportional to the biomass of each species:

$$D_a = \sum_{i=1}^{b} k_{id}B_i$$

in which k_{id} is the death rate of the i^{th} phytoplankton species.

D_i is the non-predatory death rate of the i^{th} population (see D_a)

$$D_i = k_{id}\ B_i,\ i = 1, 2, \ldots, 10.$$

D_7 is the non-predatory death rate for bacteria, assumed to be proportional to the bacterial biomass:

$$D_7 = k_{7d}\ B_7$$

in which k_{7d} is the death rate constant.

E_z is the rate of production of organic carbon (see Equation 7).

E_7 is the rate of decomposition of carbonaceous material. It is the rate of removal of carbonaceous material minus the rate of assimilation of carbonaceous material into bacterial biomass. The term for organic material x is

$$\begin{aligned} E_{7x} &= n_{xb}G_b - G_b \\ &= G_b(n_{xb}-1) \end{aligned}$$

in which n_{xb} is the stoichiometric coefficient relating moles of organic material x removed per mole of bacteria produced, and

G_b is the growth rate of bacteria (see text, BACTERIAL GROWTH).

The particular coefficients are

n_{5b} for organic carbon,

n_{6b} for organic nitrogen, and

n_{7b} for organic phosphorus

corresponding to decomposition rates E_{75}, E_{76}, and E_{77}.

G_a is the growth rate of six species of phytoplankton:

$$G_a = \sum_{i=1}^{6} \hat{\mu}_i \prod_{j=2}^{4} \left(\frac{C_j}{(K_{ij}+C_j)}\right)$$

G_i is the growth rate of the i^{th} species of phytoplankton:

$$G_i = \hat{\mu}_i \prod_{j=2}^{4} \left(\frac{C_j}{(K_{ij}+C_j)}\right)$$

For both G_a and G_i

$\hat{\mu}$ is the maximal specific growth rate for the i^{th} phytoplankton species,

C_j is the water concentration of the j^{th} nutrient, and

K_{ij} is the concentration of the j^{th} nutrient for which half maximal growth rate is attained by the i^{th} species.

G_j ($j = 8$, 9, or 10) are zooplankton growth rates (see Equation 3a ff).

G_7 is bacterial growth rate:

$$G_7 = \hat{\mu}_7 \prod_{i=5}^{7} \left(\frac{C_i}{(K_{7i}+C_i)}\right)$$

in which $\hat{\mu}_7$ is the maximal specific growth rate for bacteria, and

K_{7i} is the concentration of nutrient i for which half maximal growth rate is attained.

k_1 is reaeration rate for dissolved oxygen.

k_2 is reaeration rate for dissolved CO_2.

n_{1a} is the number of moles of O_2 produced per mole of phytoplankton produced or per mole of organic carbon secreted by phytoplankton.

n_{2a} is the number of moles of CO_2 used per mole of phytoplankton produced or per mole of organic carbon secreted by phytoplankton.

n_{3a} is the number of moles of ammonia used per mole of phytoplankton produced.

n_{4a} is the number of moles of phosphate used per mole of phytoplankton produced.

n_{5a} is number of moles or organic carbon produced per mole of algae dying from non-predatory causes or per mole of organic carbon secreted.

n_{6a} is number of moles of organic nitrogen produced per mole of algae dying from non-predatory causes.

n_{7a} is the number of moles of organic phosphorus produced per mole of algae dying from non-predatory causes.

n_{1h} is number of moles of O_2 used per mole of heterotrophic organism catabolized in respiration or per mole or organic carbon decomposed.

n_{2h} is number of moles of CO_2 produced per mole of heterotrophic organism catabolized in respiration or per mole of organic carbon decomposed.

n_{3h} is number of moles of ammonia produced per mole of heterotrophic organism catabolized in respiration or per mole of organic nitrogen decomposed.

n_{4h} is number of moles of phosphate produced per mole of heterotrophic organism catabolized in respiration or per mole of organic phosphorus decomposed.

n_{5h} is number of moles of organic carbon produced per mole of zooplankton dying from non-predatory causes.

n_{6h} is number of moles of organic nitrogen produced per mole of zooplankton dying from non-predatory causes.

n_{7h} is number of moles of organic phosphorus produced per mole of zooplankton dying from non-predatory causes.

(See also the explanation for E_7).

P_i is the predation rate on an individual of species i (see Equations 3 and 5).

R_h is the sum of the respiration rates for heterotrophs. It is assumed to be proportional to temperature per unit biomass over the temperature range used. The term is

$$R_h = \sum_{i=7}^{10} k_{iR} T B_i$$

in which k_{iR} is the respiration rate concentration ($\text{time}^{-1} \times T^{-1}$), and

T is temperature, °C.

R_j is the respiration of the j^{th} zooplankton species ($j = 8, 9,$ or 10), the j^{th} term in the sum of terms defining R_h.

R_7 is the respiration rate for bacteria, the term in the sum of terms for R_h for which $i = 7$.

S is assumed to be a constant rate per unit biomass. The term is

$$S = \sum_{i=1}^{6} k_{is} B_i$$

in which S is secretion rate for all phytoplankton combined,

k_{is} is the secretion rate constant for the i^{th} phytoplankton species, and

B_i is the biomass of the i^{th} phytoplankton species.

THE USE OF ALGAL ASSAYS TO DETERMINE EFFECTS OF WASTE DISCHARGES IN THE SPOKANE RIVER SYSTEM

William E. Miller, Joseph C. Greene, Tamotsu Shiroyama
and Ellen Merwin

INTRODUCTION

The Washington State Department of Ecology (Cunningham and Pine, 1969) and the U.S. Environmental Protection Agency, Region X (Schmidt and Kreizenbeck, 1973) defined three major water pollution problems within the Spokane-Coeur d'Alene River drainage basin. They were (1) nutrient over-enrichment, (2) toxicity (heavy metals), and (3) low dissolved oxygen.

Corrective water quality management practices have been initiated to maintain and/or obtain class A water quality standards throughout the Spokane River system, below Post Falls, Idaho, for both dissolved oxygen (8.0 mg/1) and total coliform content (medium of 240--20% not to exceed 1,000). This goal is to be met by upgrading the City of Spokane's sewage treatment plant (STP) from primary to secondary treatment. While secondary treatment would enable compliance with the class A dissolved oxygen and total coliform bacteria criteria, it would not solve the nutrient enrichment pollution problem which exists downstream from the STP.

Assessment of the nutrient enrichment problem in the Spokane River Basin is complicated by the occurrence of heavy metals (predominantly zinc) in the upper reaches of the Coeur d'Alene Lake drainage basin. Mine tailings and metallic sulfide minerals have entered the Coeur d'Alene Lake basin for over 80 years (Rabe, Wissmar, and Minter, 1973). The greatest development in mining has taken place along the south fork of the Coeur d'Alene River between Kellogg and Mullan, Idaho. In this area are located the principal mineral deposits of the Spokane Basin, including the largest silver mine and the first and third largest lead producing mines in the United States. Ores from the mines are reduced in several mills, usually located near the larger producing mines. A large lead smelter located at Kellogg, Idaho, and an electrolytic zinc plant and cadmium refinery reduces the ore and concentrates to refined metals ready for market (U.S. Department of the Army, Corps of Engineers, 1952). Eighty-three percent of the zinc entering the Spokane drainage basin is either from mining and smeltering wastewater effluents or is leached from mine tailings (Schmidt, et. al., 1973). Proposed and existing smelter wastewater treatment measures are currently being upgraded and implemented by the Bunker Hill Mining Company at Smelterville, Idaho, in an effort to solve the existing heavy metals pollution problem.

The impact of the proposed municipal and industrial wastewater treatment measures upon the growth of algae in the Spokane river system has been under considerable debate. Waste effluents are usually complicated mixtures of organic and inorganic substances. The interaction of these effluents on the growth of planktonic algae in a multiple use river system are not well defined. The effects of phosphorus, ionic strength and zinc interaction upon the growth of Selenastrum capricornutum Printz in defined inorganic culture media have been reported (Greene, Miller, and Shiroyama, 1974). Those results indicated that ionic strength, expressed as µmhos/cm can be multiplied by a factor of 2.72 to obtain the concentration of zinc (µg/l) that will inhibit growth 95% or more of the test alga; the sensitivity of S. capricornutum to zinc is inversely proportional to the ionic strength of the test substrate; and a twenty-fold increase in phosphorus content of the test water did not affect zinc toxicity.

Phosphorus and nitrogen yield relationships for S. capricornutum grown in defined inorganic culture media have been determined (Shiroyama, Miller and Greene, 1974). Factors for converting the orthophosphorus and total soluble inorganic nitrogen (TSIN = $NO_2 + NO_3 + NH_3$) content of a test substrate into maximum yield of the test alga have been determined. Each μg/l of orthophosphorus in waters containing > 10 μg P/l, when other constituents are not growth limiting, will support 0.43 mg dry wt/l of S. capricornutum. Waters containing less than 10 μg phosphorus/l generally support less than 0.10 mg dry wt per microgram of orthophosphorus. One microgram/l of TSIN in waters which are not growth limited by constituents other than inorganic nitrogen will support 0.038 mg dry wt/l of the test alga. These factors can be used to identify potential eutrophication problem sites. However, it should be recognized that constituents other than nitrogen or phosphorus may be growth limiting. The calculated theoretical productivity potential must be verified by actual algal assay analysis to determine (1) the presence of other growth limiting nutrients, (2) the presence of toxicants such as heavy metals and (3) if the chemical analyses for nitrogen and phosphorus within the test substrates are realistic.

This paper is concerned with the results of algal assays performed on water collected at seven Spokane River and tributary sites (Fig. 1) to evaluate the impact of industrial (smelter and mining) and domestic waste effluents on the growth of planktonic algae. The goals of the assay were to determine (1) the nutritional--nitrogen and phosphorus--status of the Spokane River system, (2) the critical nutrient responsible for the support of algal growth within the Spokane River system and (3) the algistatic concentration of zinc that would prevent the growth of planktonic algae.

METHODS

The Algal Assay Procedure, (AAP) Bottle Test, August 1971, using Selenastrum capricornutum Printz as the test alga, was used to assess the algal growth response of the Spokane River samples. Prior to assaying, the samples were autoclaved to solubilize the nutrients in the indigenous biomass, carbonated with a mixture of one percent carbon dioxide in air until the original pH was obtained, and filtered through

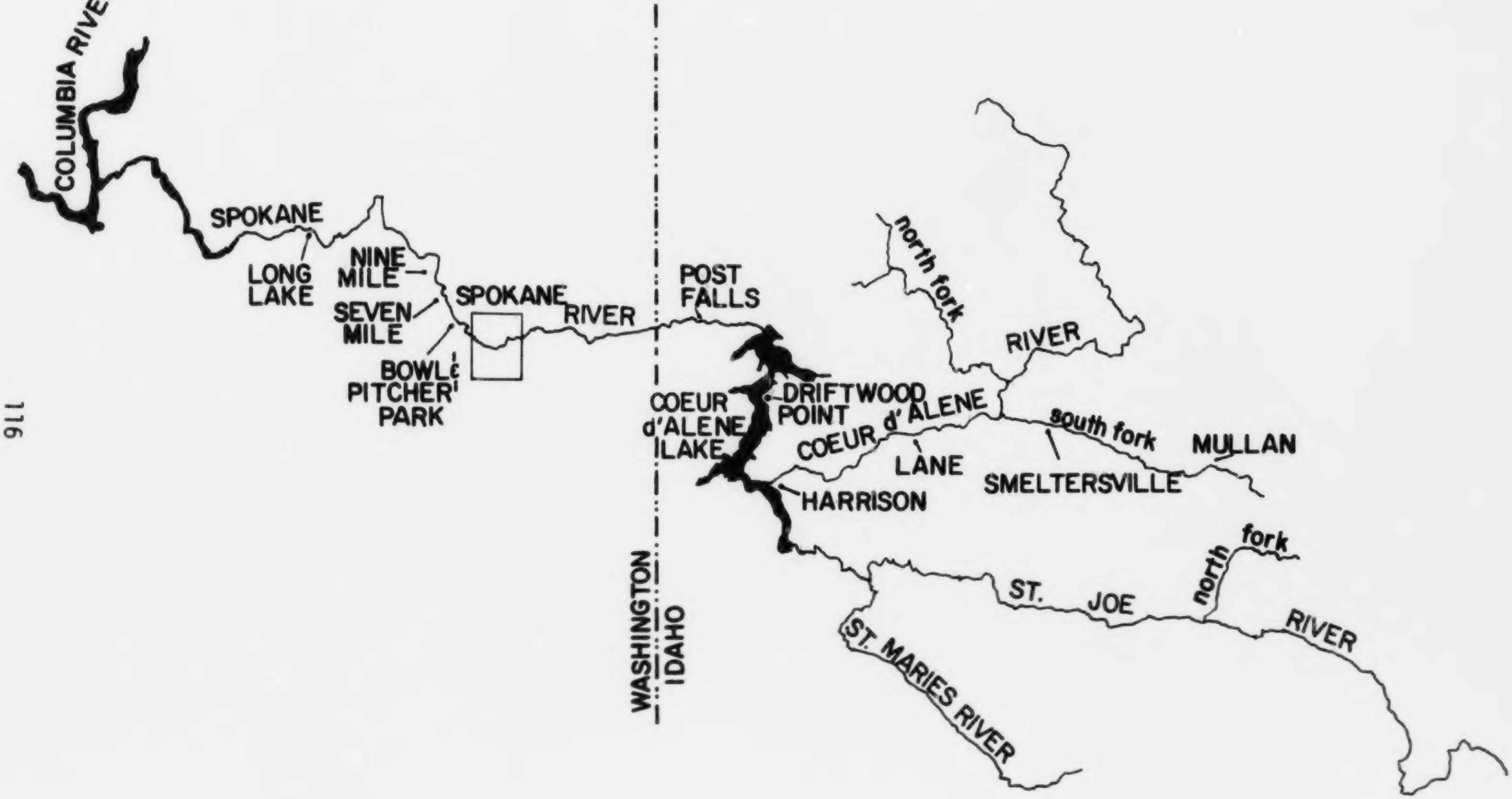

COLUMBIA RIVER
SPOKANE
LONG LAKE
NINE MILE
SEVEN MILE
BOWL & PITCHER PARK
SPOKANE RIVER
POST FALLS
WASHINGTON
IDAHO
COEUR d'ALENE LAKE
DRIFTWOOD POINT
HARRISON
COEUR d'ALENE
LANE
north fork
RIVER
south fork
SMELTERSVILLE
MULLAN
ST. JOE
north fork
RIVER
ST. MARIES RIVER

Figure 1. Spokane River Basin sampling sites

a 0.45 micron porosity membrane filter to remove particulate material which would interfere with using an electronic particle counter for measurement of algal biomass in the inoculated test samples. Results of the algal assay are reported as the maximum yield of the test alga (mg dry wt/l) obtained during a 14-day incubation period.

Seven sampling sites (Fig. 1) were chosen in the Spokane River Basin which represented varying types of water quality with respect to both nutrients and the presence of toxic materials. The water sample collected at the seven mile station in the Spokane River was divided into two aliquots; control and control passed through Dowex Al exchange resin (sodium form) to remove selected divalent and polyvalent heavy metal cations. After passage through the ion exchange resin, calcium and magnesium, equivalent to that in the original water sample, were added back to the water. A mixture of trace metals (AAP Bottle Test, 1971) was also added back to the water. Pretreatment with 1.0 mg/l ethylene diamine tetra acetic acid (EDTA) sodium salt was made prior to the assay of the water sample collected at Riverside State Park.

DISCUSSION AND RESULTS

Nutrients enter the Spokane River basin from several major sources including domestic, industrial, agricultural and groundwater intrusion. Population increases and lack of adequate wastewater treatment facilities have been identified as the major causes of increasing amounts of nitrogen and phosphorus entering the Coeur d'Alene and St. Joe River systems (Funk, *et al*., 1973).

Nitrogen trends in the Spokane River system indicate increasing nitrate concentrations due to groundwater accretions. The industries, the Spokane sewage treatment plant, and tributaries confluent with the Spokane River were relatively insignificant direct sources of nitrate nitrogen compared to the groundwater (Schmidt and Kriezenbeck, 1973).

Total soluble inorganic nitrogen levels in the natural waters of the Spokane and Coeur d'Alene drainage systems range from a low of 0.001 mg/l at Post Falls, Idaho, to a high of 0.918 mg/l at Smelterville, Idaho. A conversion factor of 0.038 multiplied by the micrograms total soluble inorganic nitrogen per liter in the test water, (Shiroyama, Miller and Greene, 1974) can be used to predict the 14-day maximum yield of S. capricornutum if nitrogen is the primary limiting nutrient and no toxic consituents are present. Theoretically, therefore, enough nitrogen is present in these waters to support 0.04 to 34.88 milligrams dry weight of the test alga during the 14-day culture period.

Miller, Maloney, and Greene, (1973) established productivity subgroups for 14-day cultures of S. capricornutum as low productivity (0.00-0.10 milligrams dry weight per liter), moderate productivity (0.11-0.80 milligrams dry weight per liter), moderately high productivity (0.81-6.00) milligrams dry weight per liter), and high productivity (greater than 6.00 milligrams dry weight per liter).

Nitrogen productivity potential within the Spokane drainage basin ranges from low to high with the greatest potential in the reach below Riverside State Park to Long Lake Dam. Control of nitrate nitrogen within the lower Spokane River basin is complicated by groundwater inflow containing high concentrations of nitrate nitrogen. The control of nitrogen productivity is also limited by economics and the presence of blue-green nuisance algae and other microorganisms capable of fixing atmospheric nitrogen. These factors led us to concentrate on phosphorus as the controlling constituent to regulate planktonic algal productivity within the Spokane River system.

The south fork of the Coeur d'Alene River at Mullan, Idaho, is a low nutrient high quality water, uncontaminated by mine waste discharge. An autoclaved and filtered sample of the south fork at Mullan, collected in November 1972, had a conductivity of 49 μmhos/cm and contained 7 μg orthophosphorus/l, 3 μg TSIN/l and 18 μg Zn/l. The orthophosphorus and inorganic nitrogen content indicated that, theoretically, it would support algal growth in amounts not greater than 0.11 mg dry wt (0.038 times 3 μg TSIN/l). The actual algal yield in the sample was 0.03 mg dry wt/l (Figure 2). The addition of 20 μg P/l to the sample produced 0.31 mg dry wt/l of S. capricornutum. The predicted algal yield for this phosphorus concentration (27 μg P/l) in the presence of all other essential nutrients is 11.6 mg (27 X 0.43) dry wt/l of the test alga. The failure of the test water to support this amount of algal growth indicated that the south fork of the Coeur d'Alene River may become limited by other essential nutrients when sufficient phosphorus is added to the test water. The addition of 1000 μg N/l and 20 μg P/l increased algal growth to 8.2 mg dry wt/l or 71 percent of the expected yield of 11.6 mg dry wt/l. This indicated that when the nitrogen and phosphorus requirements for algal growth were satisfied, either other essential nutrients or the presence of toxic materials limited maximum algal growth in the test water. The ionic strength (49 μmhos/cm) of this water multiplied by 2.72 indicated 133 μg Zn/l would inhibit algal growth greater than or equal to 95 percent. The 18 μg Zn/l contained in the test water could limit algal growth by 13 percent (Table 1) when sufficient nutrients are introduced. Thus, 84 percent (71 plus 13) of the algal yield obtained with the addition of 1000 μg N and 20 μg P can be accounted for. Interaction of constituents or the absence of other essential nutrients may be responsible for the other 15 percent of anticipated yield.

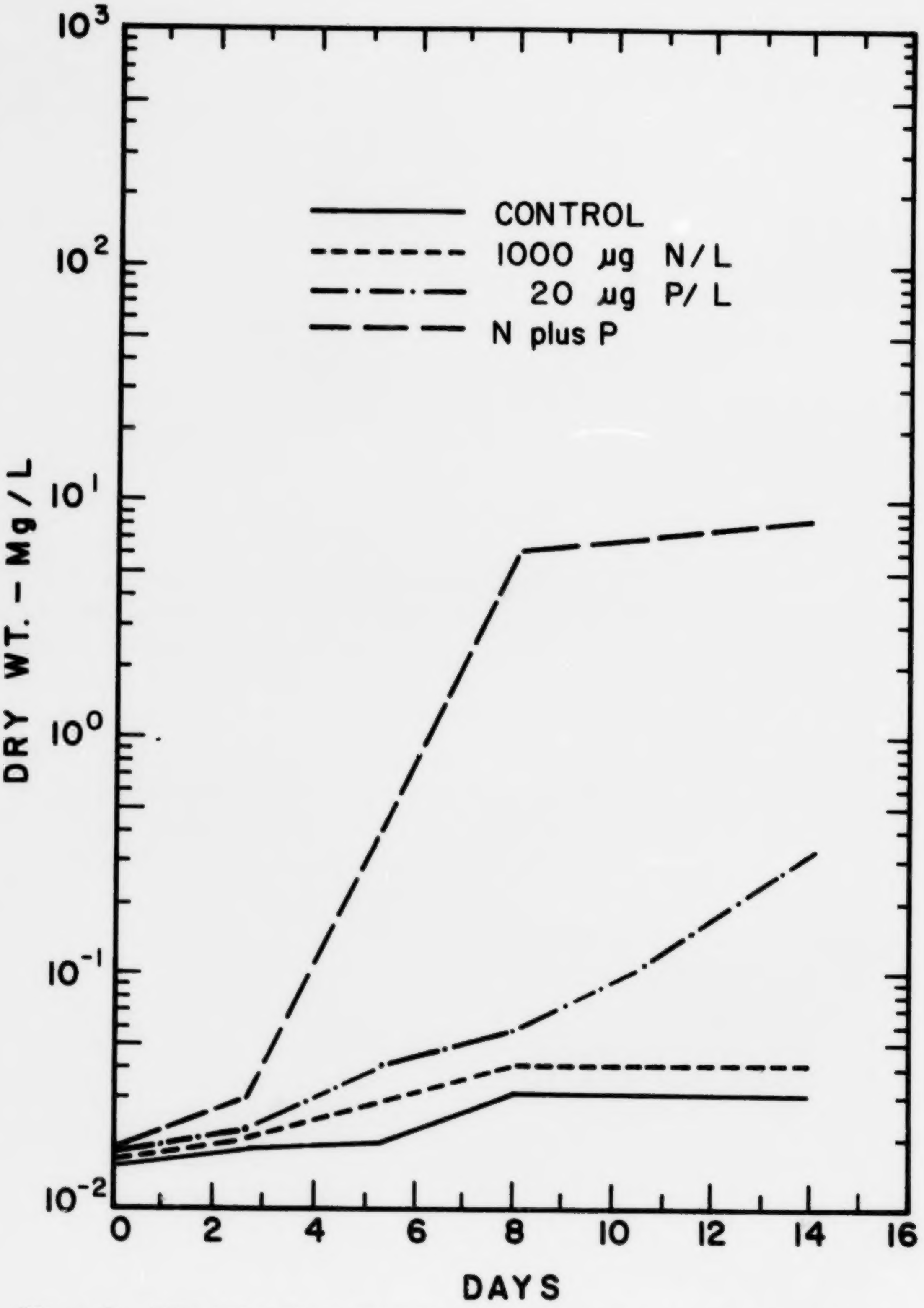


Figure 2. Effect of various nutrient additions to South Fork Coeur d'Alene River water, Mullan, Idaho on the growth of S. capricornutum

Table 1. CALCULATED PERCENT ZINC TOXICITY

Sampling Site	µg/l Zn	Specific Conductivity µmhos/cm	% calculated* Zn. toxicity
So. Fork Coeur d'Alene at Mullan	18	49	13
So. Fork Coeur d'Alene at Smelterville	7500	382	>100
Coeur d'Alene at Lane	2470	152	>100
Coeur d'Alene Lake (driftwood pt.)	118	54	80
Spokane River at Post Falls	107	49	80
Spokane River at Seven Mile Bridge	75	163	17
Spokane River at Long Lake Dam	18	169	4

*µg/l Zn ÷ (2.72 x µmhos/cm x .95) This calculation is valid for zinc only and does not take into account interaction with other heavy metals.

The Coeur d'Alene Lake watershed is the main contributor of zinc to the Spokane River; contributing about 83 percent of the total monthly load during September 1972 low flow conditions (Schmidt and Kreizenbeck, 1973).

Samples collected at the south fork of the Coeur d'Alene River at Smelterville, the Coeur d'Alene River at Lane, and Coeur d'Alene Lake (off Driftwood Point) in November 1972 contained concentrations of dissolved zinc ranging from 7500 µg/l at Smelterville to 118 µg/l off Driftwood Point. The algal assay growth responses of these waters reflects the calculated toxicity of zinc on the growth of S. capricornutum (Table 1). Each of these autoclaved and filtered test waters supported less than 0.10 mg dry wt/l of the test alga. This growth response was within the anticipated yield based on the orthophosphorus content in the samples collected at the Coeur d'Alene River at Lane and at Coeur d'Alene Lake (off Driftwood Point). The sample collected at Smelterville contained 96 µg P/l. This concentration of phosphorus, in the presence of all other essential nutrients and the absence of toxic materials, should have supported 41.3 mg dry wt/l of S. capricornutum. This theoretical (96 times 0.43) 41.3 mg dry wt/l maximum yield (calculated for the south fork of the Coeur d'Alene River at Smelterville) indicates that this water could have a high phytoplankton growth productivity potential if its zinc content (7500 µg Zn/l) is reduced below the algistatic level. An abundance of periphytic algal growth was observed in the River during the November 1972 sampling. This suggests to the authors that these periphyton may have a greater tolerance to high concentrations of zinc than phytoplankton.

The autoclaved and filtered (November 1972) water sample collected from the Spokane River at Post Falls contained 8 µg P/l, 1 µg TSIN/l and 107 µg Zn/l. The nitrogen and phosphorus nutrient content of this water is essentially the same as that found in the autoclaved and filtered Coeur d'Alene Lake (off Driftwood Point) water sample. Water collected at both sampling sites exhibited similar algal growth response to the addition of nitrogen and phosphorus (Figure 3). The average dissolved zinc (112 µg/l) in these water samples could override the effect of additional phosphorus loading, up to 20 times (160 µg P/l) greater than that presently found, upon the growth of planktonic algae (Greene et al, 1974). This suggests that zinc toxicity may be the predominant growth limiting factor for planktonic algae in the Spokane River between Post Falls and Nine Mile Bridge.

The Spokane River samples collected at Riverside State Park and Seven Mile Road bridge reflect the nutrient loading of the City of Spokane's sewage treatment plant (STP). The Spokane STP is the major contributor of phosphorus to the Spokane River. Cunnningham and Pine (1969) cited an increase from 54 kilograms orthophosphorus per day (120 pounds per day) in the upstream water to 1418 kilograms per day (3100 pounds per day) immediately downstream from the STP. This is approximately a 26-fold increase of orthophosphorus in the receiving water.

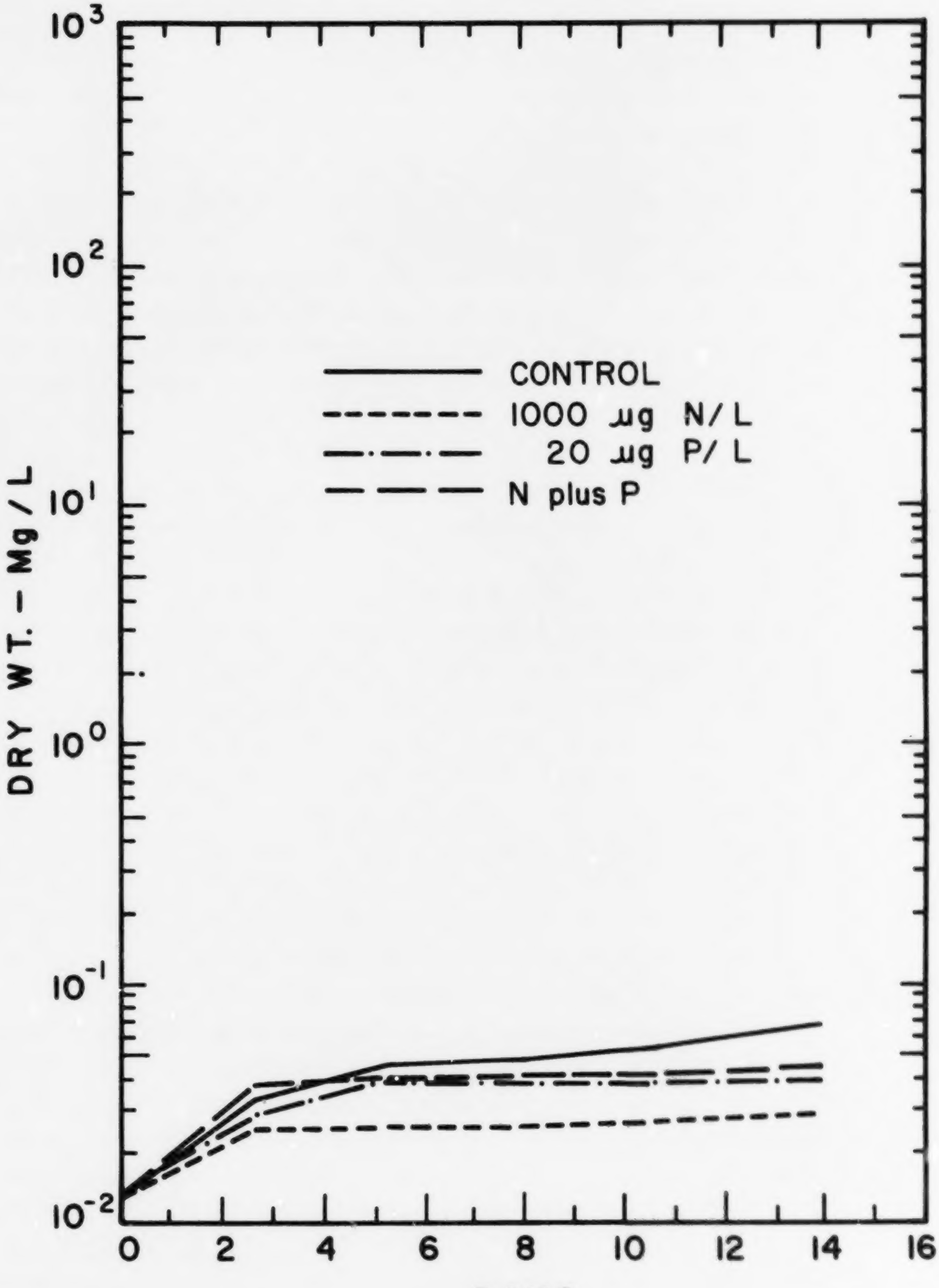


Figure 3. Effect of various nutrient additions to Spokane River water, Post Falls, Idaho on the growth of S. capricornutum

In September 1972 the Spokane Sewage Treatment Plant contributed 57 percent of the total monthly phosphorus loading to the Spokane River (Schmidt, et al, 1973).

The maximum 14-day algal yield obtained in the November 1972 (autoclaved and filtered) Spokane River water sample collected at Seven Mile Road bridge was 0.11 mg dry wt/l of S. capricornutum (Figure 4). This was 0.3 percent of the expected yield of 36.6 mg dry wt/l that could be supported by 85 μg P/l. The predicted yield for this phosphorus level is valid when either no toxicant is present in the water or when other nutrients are not growth limiting. The November 1972, Seven Mile Road bridge sample contained 75 μg/l of dissolved zinc. This level of zinc could limit algal growth 17 percent providing interaction with other heavy metals does not contribute to the overall toxicity.

Figure 5 shows the effect of removing the 75 μg Zn/l from the Seven Mile Road bridge sample (by cation exchange) upon the growth of the test alga. Algal growth yield in the treated sample was increased to 15.4 mg dry wt/l. This represents 80 percent of the theoretical yield of 19.3 mg dry wt/l calculated for the 510 μg/l TSIN content of the test water. Zinc removal from the test water increased productivity approximately 160 fold. The addition of 40 and 100 μg Zn/l back to the metal stripped sample again inhibited algal growth.

The effect upon zinc toxicity of the addition of 1.0 mg EDTA/l to an autoclaved and filtered Riverside State Park water sample was also evaluated. This water sample contained 115 μg P/l, 509 μg TSIN/l and 125 μg Zn/l. The yield in the untreated sample was 0.12 mg dry wt/l of S. capricornutum. This represents less than 1 percent of the expected yield of 19.4 mg dry wt/l based on the growth limiting TSIN content of the test water (Figure 6). After chelation with 1.0 mg EDTA/l this test water supported 21.7 mg dry wt/l of the test alga. These data indicate that zinc and other heavy metals are primarily limiting planktonic algal growth within the Spokane River system. This also reinforces our hypothesis that zinc interaction downstream from the Spokane sewage treat-

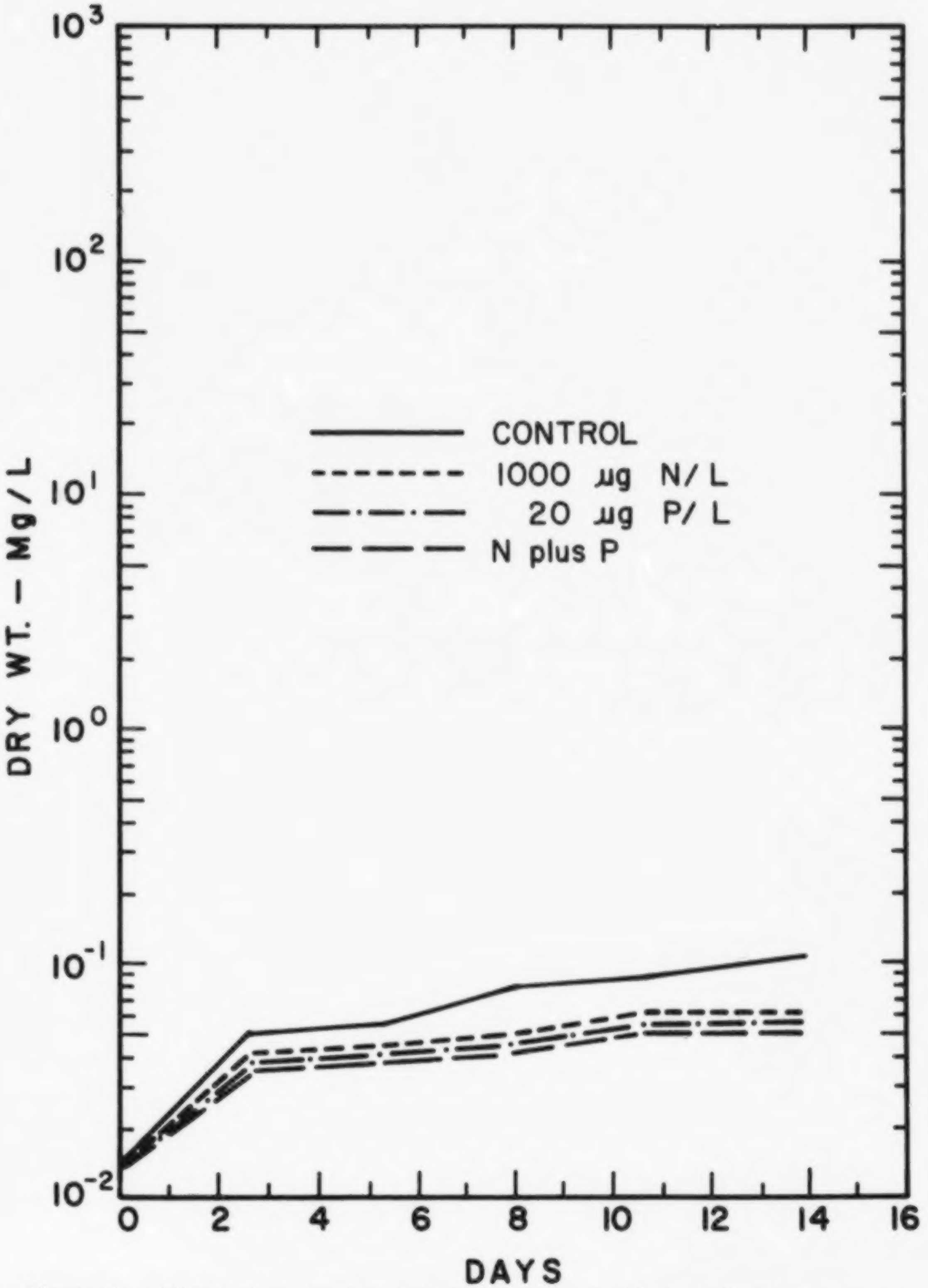


Figure 4. Effect of various nutrient additions to Spokane River water, Seven Mile Road Bridge, Washington on the growth of S. capricornutum

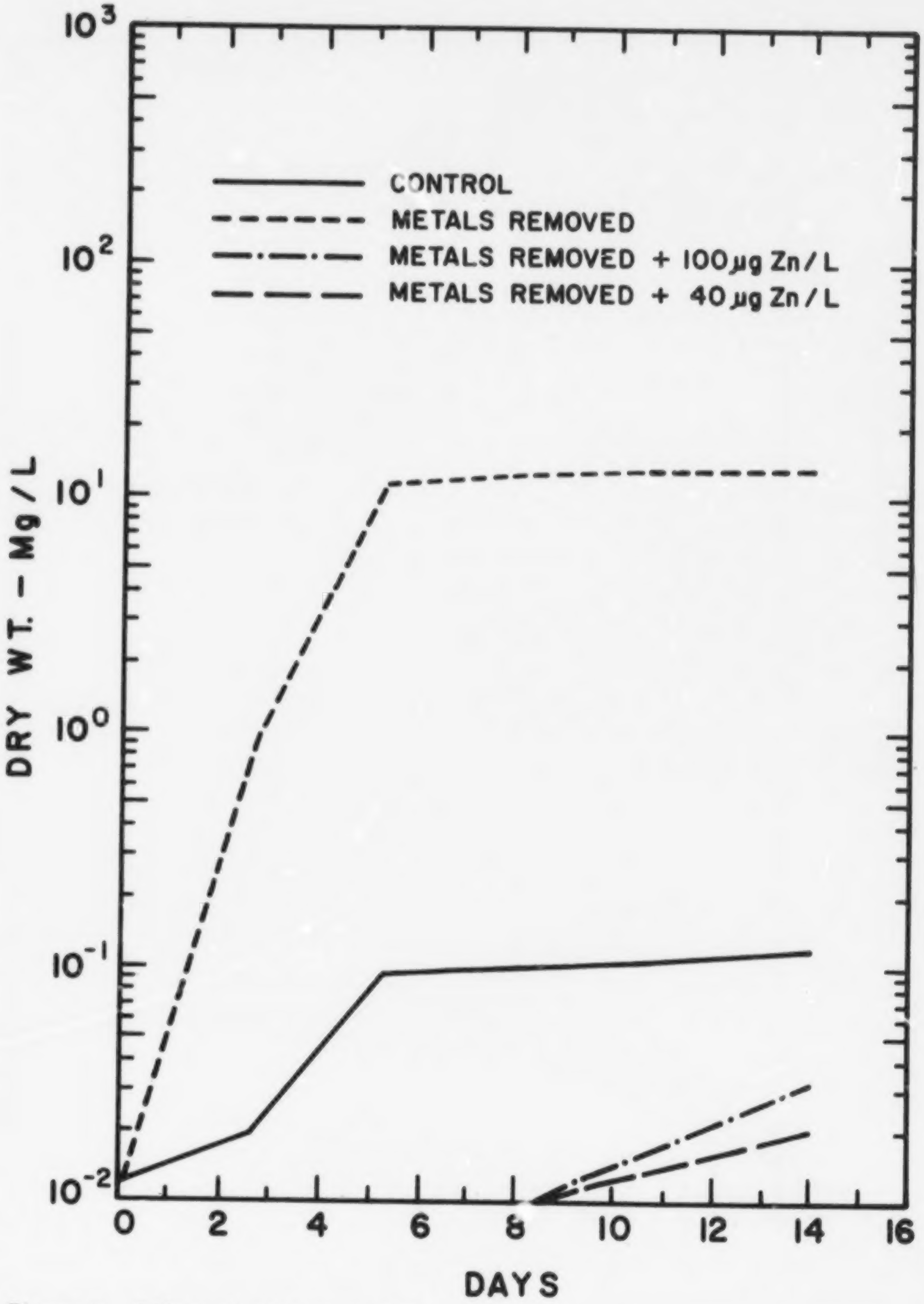


Figure 5. Effect of zinc removal from Seven Mile Road Bridge water on the growth of S. capricornutum

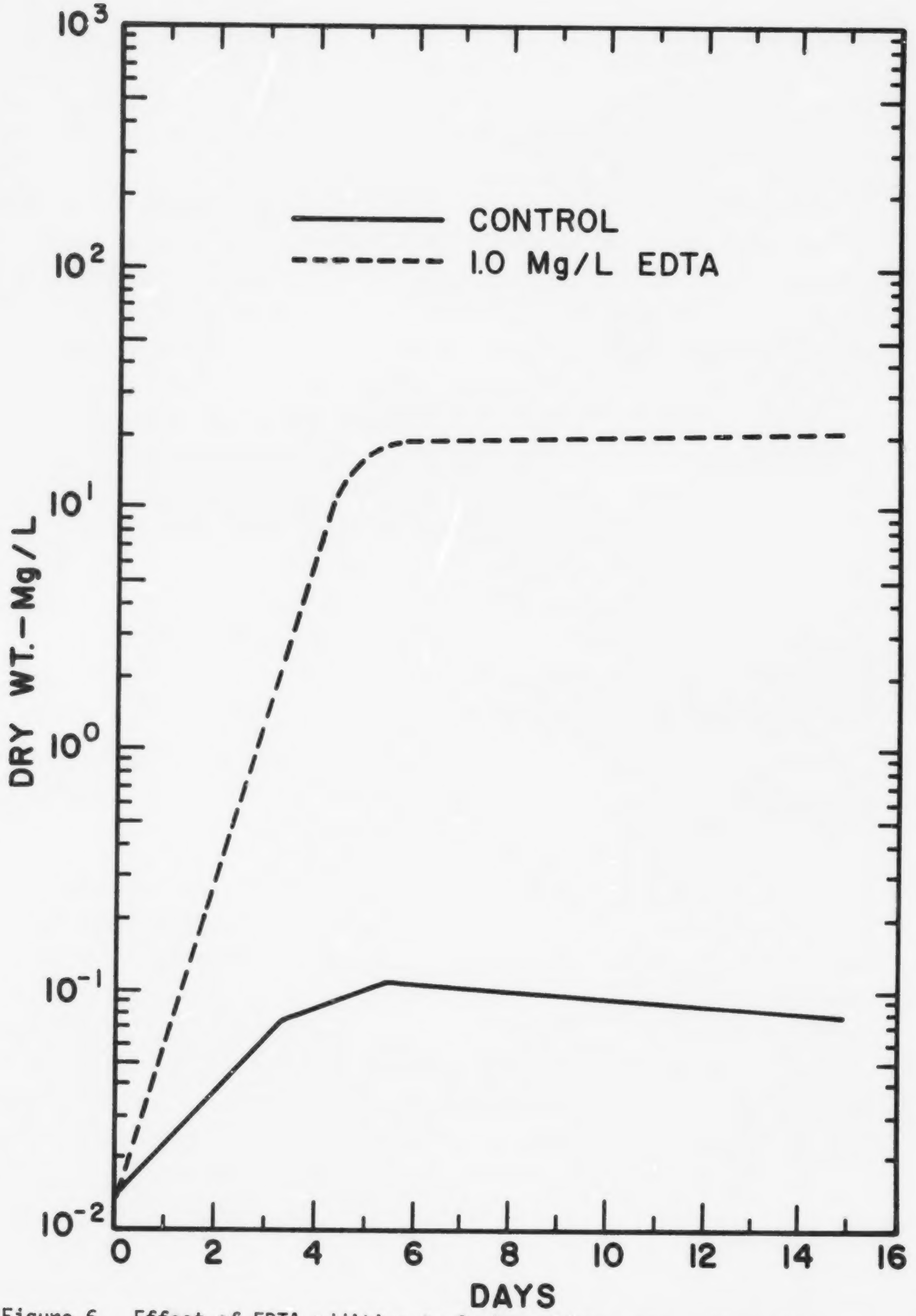


Figure 6. Effect of EDTA addition to Spokane River water, Riverside State Park, Washington on the growth of S. capricornutum

ment plant prevents massive algal growth relative to the nutrient loading from the Spokane sewage treatment plant.

Natural decomposition, and/or complexing of zinc by organic compounds or some other Zn removal mechanism, downstream from the Spokane sewage treatment plant reduced the concentration of zinc from an average of 112 μg/l immediately downstream from the treatment plant to less than 20 μg Zn/l in the (November 1972) Long Lake Dam water sample. The growth response of S. capricornutum, reflecting the reduced zinc level within the Long Lake Dam water sample, is shown in Figure 7. The maximum yield obtained in this sample was 14.9 mg dry wt/l. The theoretical yield, based on the orthophosphorus content of 46 μg/l, is 19.8 mg dry wt/l. The lower than anticipated growth response to the addition of nitrogen and phosphorus indicates that either some essential nutrient other than phosphorus may be limiting maximum algal growth or that a toxic substance, other than zinc, may be present in this water. However, a growth response of 14.9 mg dry wt/l of S. capricornutum in the algal assay indicates that Long Lake is a highly productive body of water.

SUMMARY AND CONCLUSIONS

Algal assays were used to define the effects of heavy metal and domestic wastewater effluents upon the potential growth of planktonic algae within the Spokane River Basin. Results of these assays led to the following conclusions:

(1) Nitrogen and phosphorus additions stimulated algal growth in waters collected in the upper reaches of the south fork of the Coeur d'Alene River at Mullan, Idaho. Stimulatory effects of these nutrients decreased proportional to the zinc concentrations in waters collected downstream from Smelterville, Idaho.

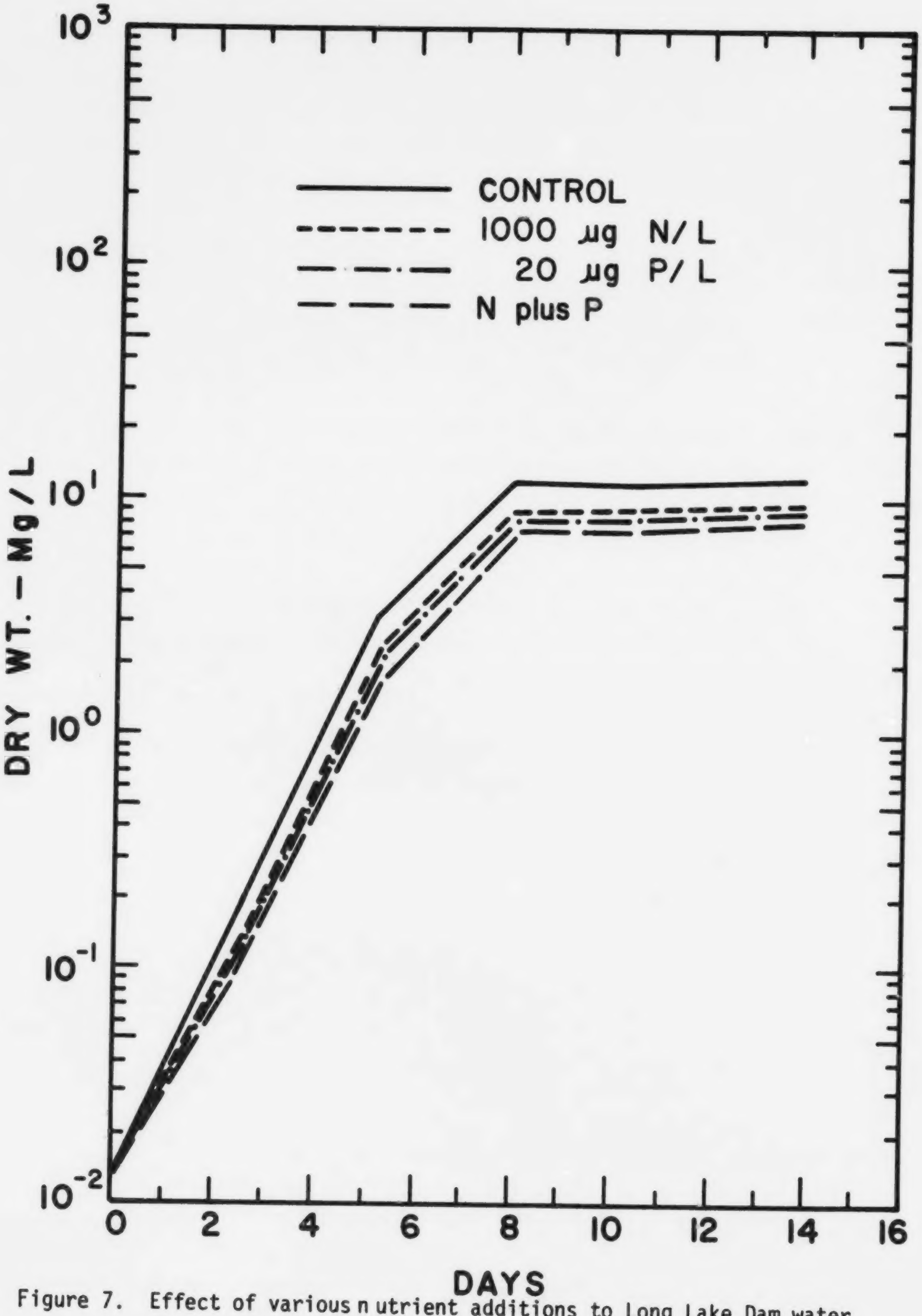


Figure 7. Effect of various nutrient additions to Long Lake Dam water on the growth of S. capricornutum

(2) Algal growth potential in the Spokane River from Post Falls, Idaho, to Riverside State Park, Washington, is regulated by the average dissolved zinc content of 112 μg/l.

(3) A 20-fold increase in orthophosphorus loading to the Spokane River system upstream from Riverside State Park would have little effect upon the growth of planktonic algae unless the zinc content of these waters is reduced.

(4) A natural reduction of zinc from 112 μg/l at the Spokane STP to 20 μg/l at Long Lake Dam, 23 kilometers downstream from the treatment plant, enabled algal growth to increase proportionally to the orthophosphorus content of the water.

(5) Assessment of nutrient enrichment problems, complicated by the occurrence of heavy metals (predominantly zinc), can be accomplished through use of algal assays.

(6) The addition of chelators (EDTA) and the use of cation exchange resins to remove heavy metal toxicity from test waters prior to algal assay holds considerable promise. These two pretreatment methods gave similar assay results on waters collected within the Spokane River system.

REFERENCES

Cunningham, Richard K. and Roland E. Pine. 1969. A Preliminary Investigation of the Low Dissolved Oxygen Concentrations That Exist in Long Lake Located Near Spokane, Washington. Washington State Water Pollution Control Commission. Technical Report No. 69-1.

Environmental Protection Agency. 1971. Algal Assay Procedure Bottle Test. National Eutrophication Research Program, National Environmental Research Center, Corvallis, Oregon. 82 p.

Funk, William H., Fred W. Rabe, Royston Filby, Jon I. Parker, James E. Winner, Larry Bartlett, Nancy L. Savage, Paul F. X. Dunigan, Jr., Neil Thompson, Richard Condit, Paul J. Bennett, and Kishor Shah. 1973. Biological Impact of Combined Metallic and Organic Pollution in the Coeur d'Alene-Spokane River Drainage System. Washington State University and University of Idaho, Joint Report (B-044 Wash and B-015 IDA).

Greene, Joseph C., William E. Miller, Tamotsu Shiroyama, and Ellen Merwin. 1974. Variation of Zinc Toxicity to the Green Alga Selenastrum capricornutum Printz As a Function of Phosphorus or Ionic Strength. (In preparation)

Miller, William E., Thomas E. Maloney and Joseph C. Greene. 1973. Algal Productivity in 49 Lakes as Determined by Algal Assays. Water Research 8:667-679.

Rabe, Fred W., R. C. Wissmar, and R. F. Minter. 1973. Plankton Populations and Some Effects of Mine Drainage on Primary Productivity of the Coeur d'Alene River, Delta, and Lake. Water Resources Research Institute University of Idaho, Technical Report A-030-IDA.

Schmidt, Bill and Ron Kreizenbeck. 1973. September 1972 Spokane River Basin Survey Report. Presented at Spokane Feb. 20, 1973.

Shiroyama, Tamotsu, William E. Miller, and Joseph C. Greene. 1974. A Method for Predicting the Maximum Yield of Selenastrum capricornutum Printz in Phosphorus and Nitrogen Limited Waters. (In preparation)

U. S. Department of the Army, Corps of Engineers. 1952. Columbia River Basin Study.

EFFECT OF NITROGEN AND PHOSPHORUS ON THE GROWTH OF *SELENASTRUM CAPRICORNUTUM*

Tamotsu Shiroyama, William E. Miller
and Joseph C. Greene

INTRODUCTION

Phosphorus and nitrogen have been established as the two most critical nutrients related to algal productivity and eutrophication. In 1919, E. Naumann indicated that phosphorus and nitrogen play a major role in the eutrophication process. More recently, Vollenweider (1968) reiterated Naumann's viewpoint. Sawyer (1968), Weiss (1969), Miller *et. al*. (1971, 1973), and others have also shown that phosphorus and nitrogen are important in algal productivity. This paper discusses the growth stimulating and/or limiting effects of phosphorus and nitrogen upon *Selenastrum capricornutum* Printz. cultured in a defined medium.

METHOD

The Algal Assay Procedure Bottle Test (EPA, 1971) was used exclusively throughout this investigation. The assays were carried out in 500-ml Erlenmeyer flasks containing 100 ml of the algal assay medium (AAM). Dibasic potassium phosphate (K_2HPO_4) and sodium nitrate ($NaNO_3$) salts were used as sources of phosphorus and nitrogen respectively. All assays were conducted in triplicate and each flask was inoculated from a seven-day-old culture of *S. capricornutum* grown in AAM to give a final concentration of 1000 cells/ml. Inoculated flasks, without the added phosphorus or nitrogen, served as controls.

The cultures were incubated at 24±2°C under continuous "cool-white" fluorescent lighting of 400 ft-c (4304 lux) and continuously shaken at 110 oscillations per minute for a maximum of 14 days. Counts were made with an electronic particle counter equipped with a mean cell volume computer. Algal cell were counted on days 3, 5, 7, 10, and 14.

RESULTS AND DISCUSSION

Phosphorus

By maintaining the nitrogen concentration at 4.200 mg/l (nitrogen concentration provided in AAM), the response of S. capricornutum showed that, when nitrogen was not growth-limiting, the maximum yield increased proportionally with each phosphorus addition. This relationship between the biomass (mg/l dry wt.) produced with each concentration of phosphorus as a function of time (days) is shown in Figure 1. A linear relationship exists between maximum yield (mg/l) and phosphorus concentration (Figure 2) up to a phosphorus concentration of approximately 0.300 mg/l. Above this concentration, a nutrient other than phosphorus becomes growth-limiting. These results show that AAM, having a phosphorus concentration of 0.186 mg/l, is definitely phosphorus limiting for the growth of S. capricornutum. Also, an increase or decrease in the concentration of phosphorus concomitantly affects the maximum growth rate. For example, by the fifth day at which the growth rate was at its maximum, the phosphorus increase from 0.006 to 0.012, to 0.023, to 0.046, to 0.092, to 0.186 mg/l resulted in a maximum growth rate increase from 0.871 to 1.07, 1.19 to 1.31, and to 1.42 day^{-1}, respectively.

A linear regression analysis of phosphorus versus algal biomass (mg/l dry wt.) showed that on days 3, 5, 7, 10, and 14, the correlation between the two variables did not become significant until day 5 (Figure 3). This same statistical analysis also demonstrated that the growth

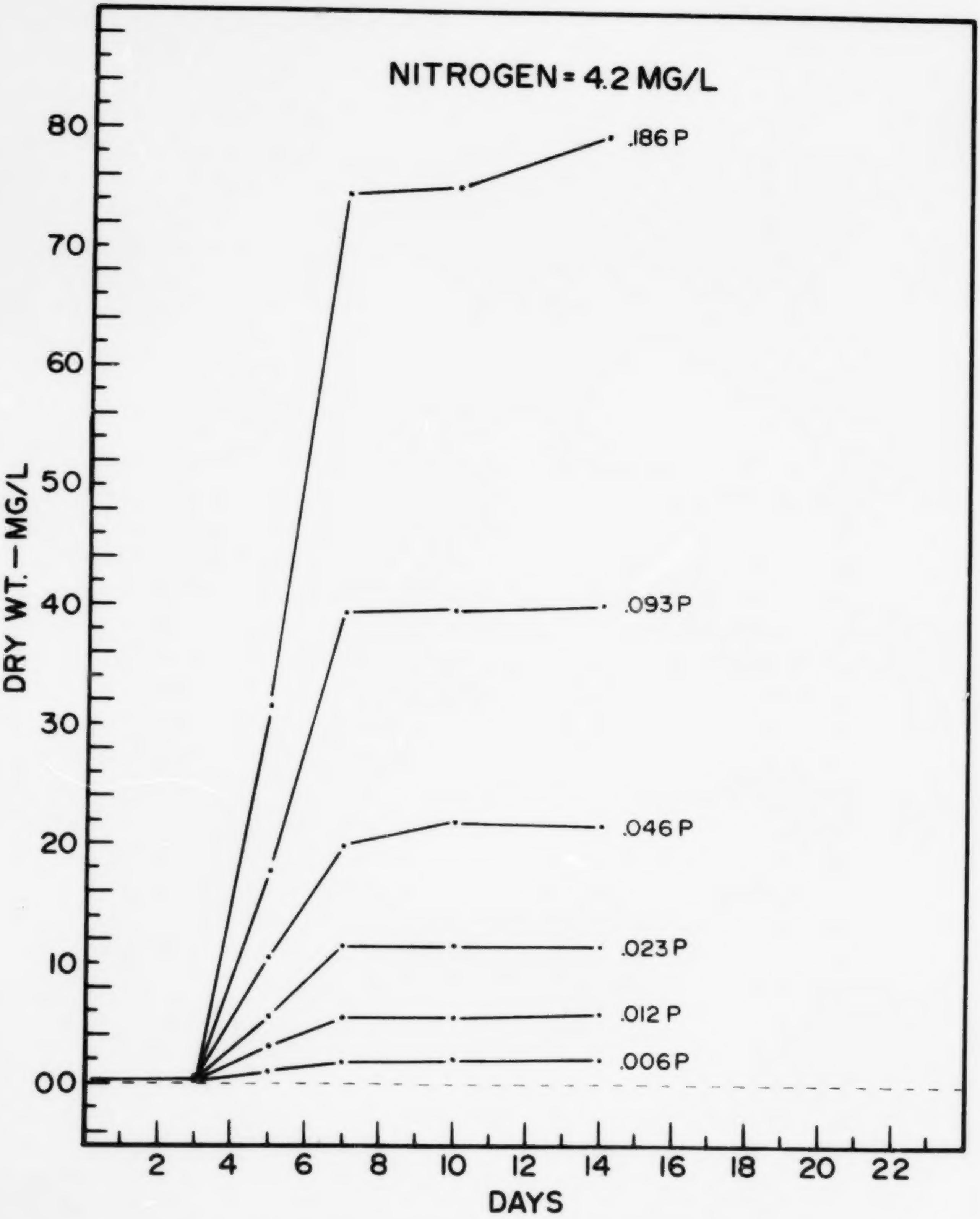


Figure 1. Effect of phosphorus additions in AAM on the growth of *S. capricornutum*

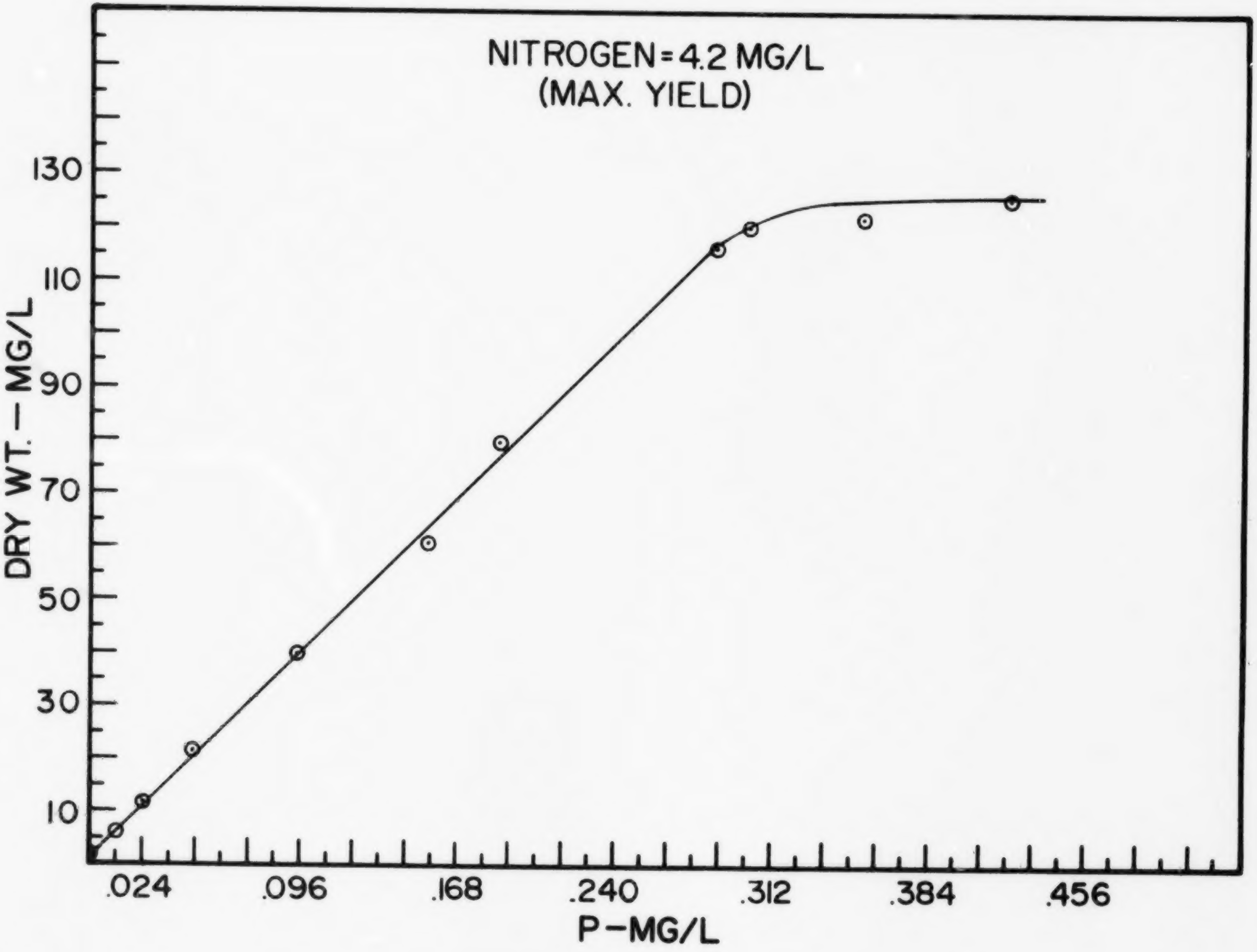


Figure 2. Maximum yield of S. capricornutum in AAM with phosphorus additions

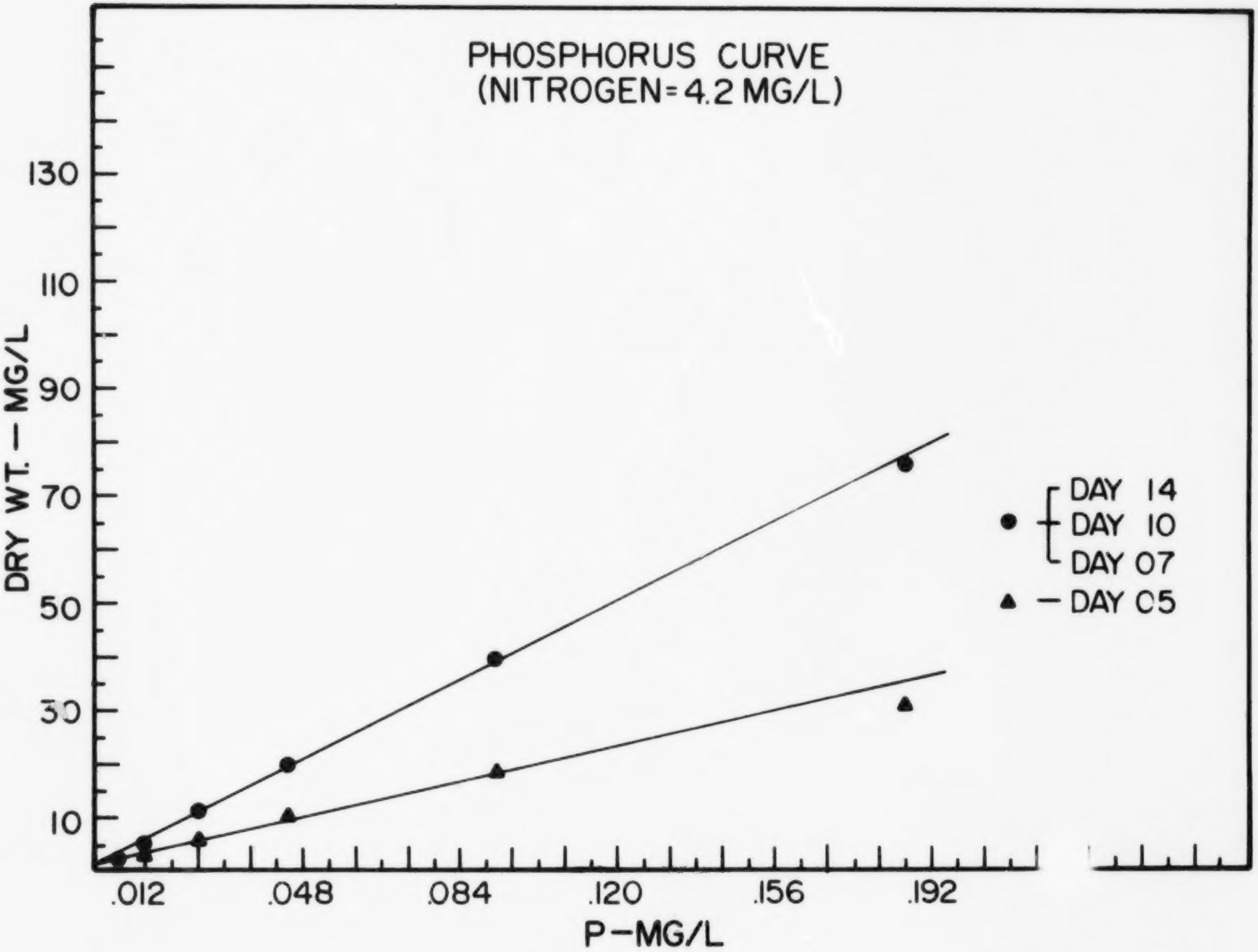


Figure 3. The relationship of biomass and phosphorus addition in AAM of S. capricornutum

responses for days 7, 10, and 14 were nearly identical and the correlation coefficients between phosphorus and biomass for the five different days were 0.622, 0.995, 0.999, 0.998, and 0.999, respectively.

The linearity of the phosphorus curve (Figure 2) makes it possible to utilize the slope in predicting the maximum yield of the alga. In the presence of other essential nutrients and in the absence of any toxicants, .001 mg/l of orthophosphorus in water containing $\geq$ .010 mg P/l will yield 0.43 mg/l dry weight of the alga.

Nitrogen

The effect of nitrogen upon the growth of S. capricornutum was also evaluated. The phosphorus concentration was maintained at the level used in AAM (0.186 mg/l) and the nitrogen concentrations were varied from 0.016 to 4.200 mg/l (Figure 4). A comparison of the growth rates found during the phosphorus additions (Figure 1) and by the nitrogen additions (Figure 4) indicate that these two nutrients do produce similar results. For example, the maximum growth rate for nitrogen was achieved by the seventh day, but during phosphorus addition this rate was achieved by the fifth day. When the nitrogen was increased from 0.016 to 0.064 to 0.132 to 0.524 to 2.10 mg/l, the growth rate increased correspondingly from 0.496 to 0.613 to 0.778 to 0.977 and to 1.034 day^{-1}, respectively.

The t-test distribution analysis on the maximum algal yield showed that with lower concentrations of nitrogen the yield did not become significant until nitrogen levels of between 0.032 and 0.064 mg/l were reached (Figure 5). At concentrations of nitrogen greater than 2.10 mg/l, with phosphorus being the limiting growth factor (0.186 mg/l), the maximum yield became less significant.

The linear regression analysis showed, as it did with phosphorus, a definite linear relationship between maximum yield and nitrogen concen-

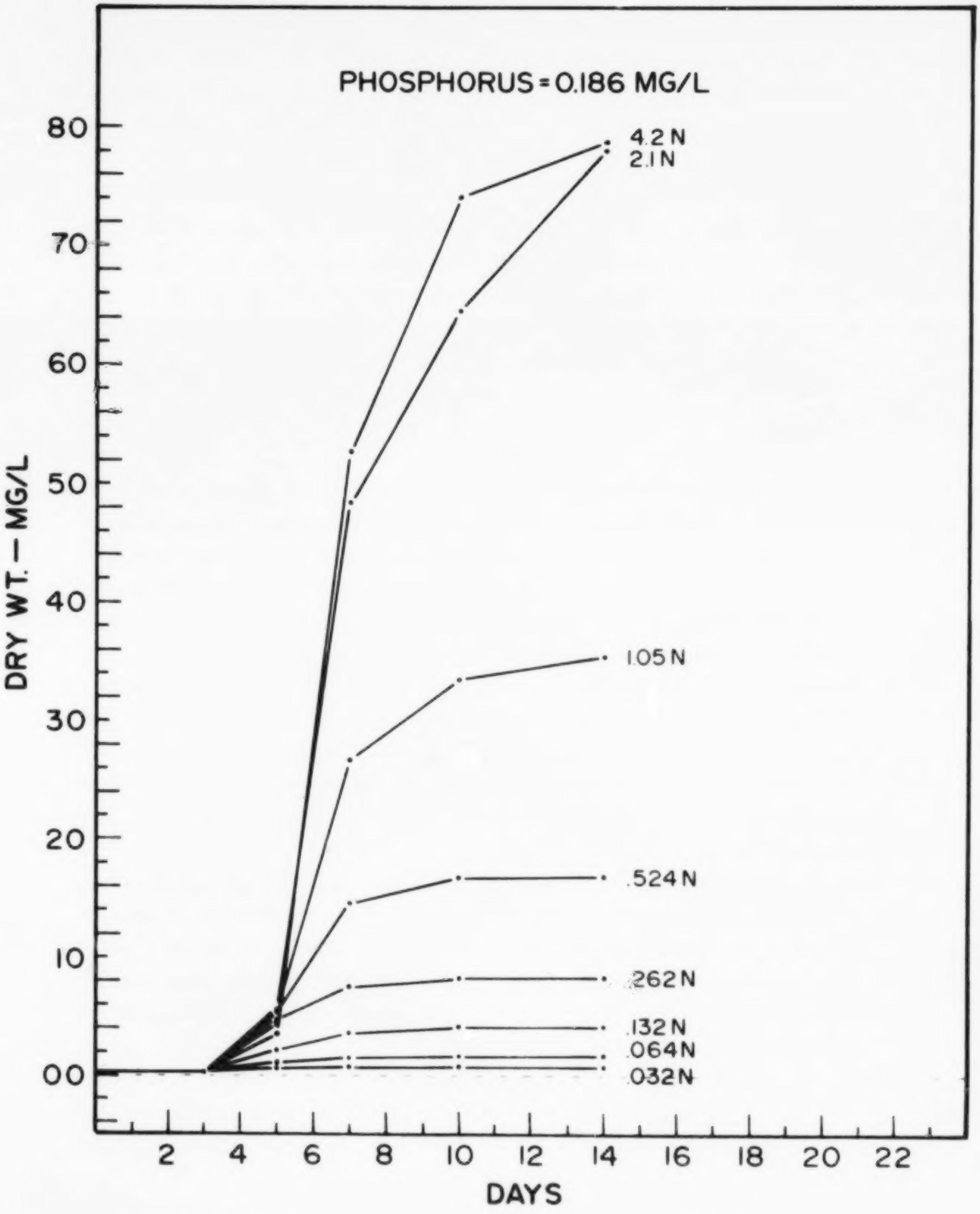


Figure 4. Effect of nitrogen additions in AAM on the growth of S. capricornutum

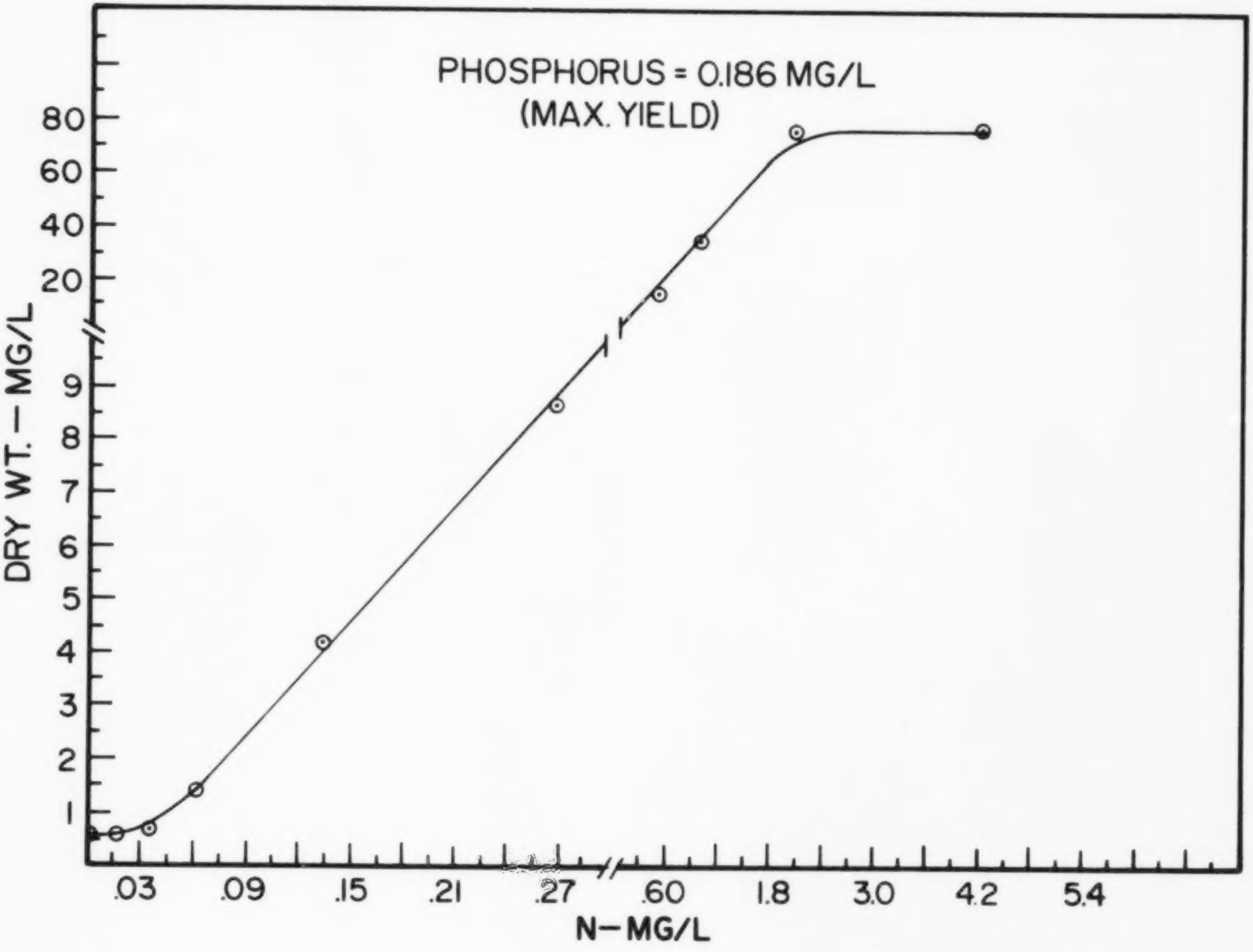


Figure 5. Maximum yield of S. capricornutum in AAM with nitrogen additions.

tration (Figure 5). This relationship was linear at nitrogen concentrations of 0.032 to 2.10 mg/l, above which, the phosphorus concentration became growth-limiting thereby preventing further utilization of nitrogen by the algal cell. Figure 6 shows the linear relationship for days 5, 7, 10, and 14 where the correlation coefficients between nitrogen and biomass (mg/l) were 0.949, 0.998, 0.999, and 0.999, respectively.

Figure 5 was used for ascertaining the predictive yield factor for nitrogen. Assuming the essential nutrients and toxic conditions stated earlier for orthophosphorus, .001 mg/l of total soluble inorganic nitrogen (NO_2+NO_3+NH_3) will produce a maximum yield of .038 mg/l dry weight of the alga.

CONCLUSION

Growth rates of S. capricornutum, grown in Algal Assay Medium, revealed that the major uptake of phosphorus and nitrogen occurred in the first five days of growth.

The higher growth rate observed with phosphorus, as compared to nitrogen indicates that the test alga assimilates phosphorus more rapidly than nitrogen.

There is a definite linear relationship between biomass produced and the amount of phosphorus and nitrogen present.

The determination of phosphorus and nitrogen requirements for the alga, indicate that when all other essential nutrients are present and in the absence of any toxicants, the maximum growth responses of the alga can be predicted. Waters containing ≥ .010 mg/l orthophosphorus will yield 0.43 milligrams dry weight of the alga per .001 mg P/l. Similarily for each .001 mg/l total soluble inorganic nitrogen will yield .038 milligrams dry weight of the alga. Actual yield is considered statistically significant within ±20 percent of the predicted yield.

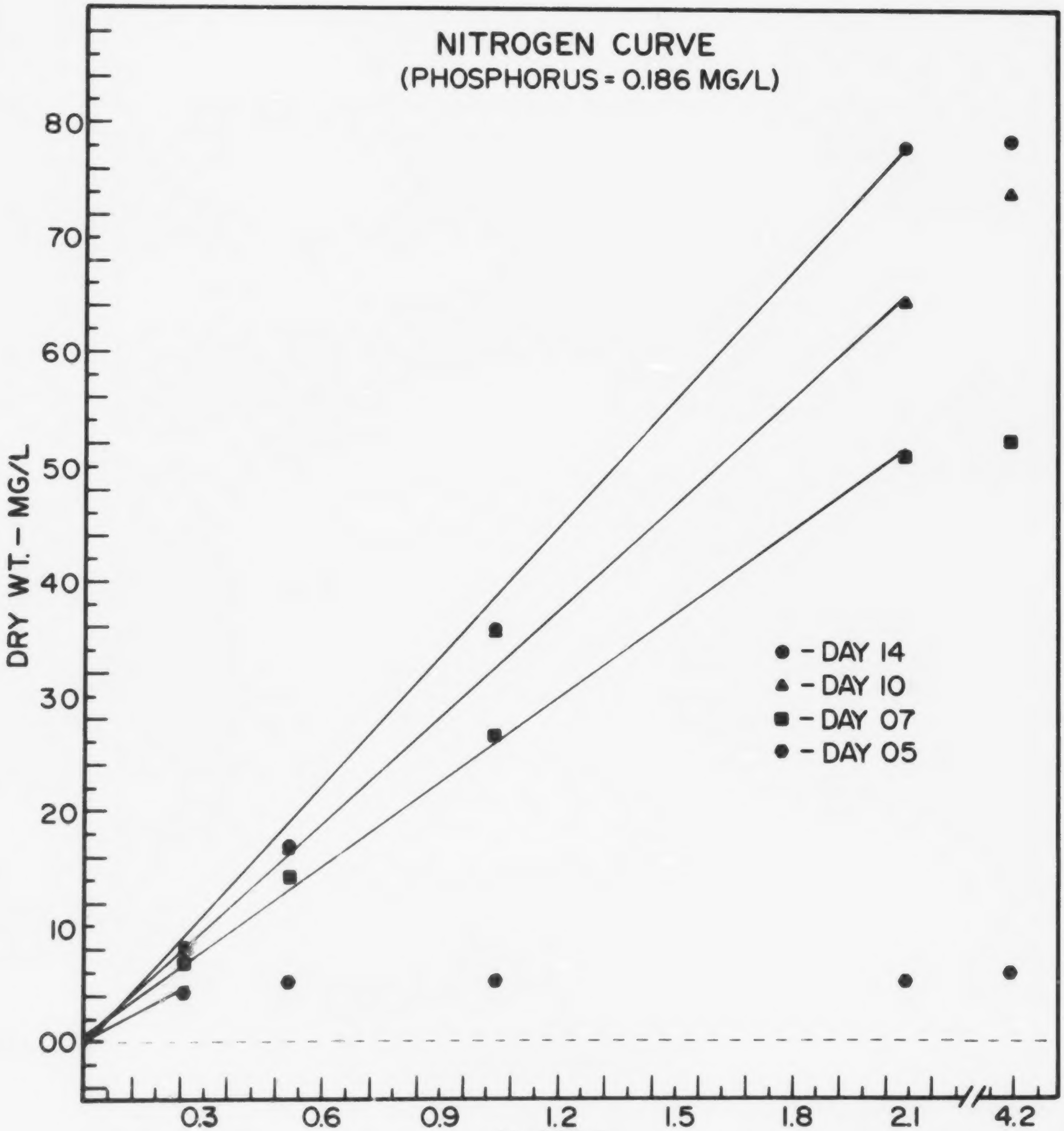


Figure 6. The relationship of biomass and nitrogen additions in AAM of *S. capricornutum*

REFERENCES

Environmental Protection Agency. 1971. Algal Assay Procedure: Bottle Test. National Eutrophication Research Program, National Environmental Research Center, Corvallis, Oregon.

Miller, W. E., T. E. Maloney, and J. C. Greene. 1973. Algal Productivity in 49 Lake Waters as Determined by Algal Assays. (In Press)

Miller, W. E. and T. E. Maloney. 1971. Effects of Secondary and Tertiary Sewage Effluents on Algal Growth in Waters of a Lake-River System. J. Water Poll. Con. Fed. 43(12):2361-2365.

Naumann, E. 1919. Nagna Synpunkter Agaenda Limnoplanktons Okologie. Svensk. Bot. Tidsker. 13:129-163.

Sawyer, C. N. 1966. Basic Concepts of Eutrophication. J. Water Poll. Con. Fed. 38(5):737-744.

Shapiro, J. and R. Ribeiro. 1965. Algal Growth and Sewage Effluent in the Potomac Estuary. J. Water Poll. Con. Fed. 37(7):1034.

Vollenweider, R. A. 1968. Scientific Fundamentals of the Eutrophication of Lakes and Flowing Waters, with Particular Reference to Nitrogen and Phosphorus as Factors in Eutrophication. Technical Report to the Organization for Economic Cooperation and Development, Committee for Research Cooperation. 159 p.

Weiss, C. M. 1969. Relation of Phosphates to Eutrophication. J. Amer. Water Works Assoc. 61(8):387-391.

THE USE OF IN SITU ALGAL ASSAYS TO EVALUATE THE EFFECTS OF SEWAGE EFFLUENTS ON THE PRODUCTION OF SHAGAWA LAKE PHYTOPLANKTON

Paul D. Smith

INTRODUCTION

Shagawa Lake is a eutrophic lake in northeastern Minnesota near the Canadian Border. It has an area of about 9.2 km^2, a mean depth of 5.7 m and a maximum depth of 13.7 m. About 65% of the surface inflow to the lake is from the Burntside River which drains oligotrophic Burntside Lake; the remainder comes from several minor streams (Malueg, et al. 1973). Because the region is primitive geologically and its lakes are predominantly oligotrophic, Shagawa Lake contrasts sharply by being one of the more productive water bodies in Minnesota (Megard 1970). Paleolimnetic studies (Bradbury and Waddington, 1973) indicate the lake is a victim of cultural eutrophication as a result of past logging and mining on its shores and by virtue of its function over the past 70 years as a receptacle for the domestic wastes of the city of Ely (pop. 5,000).

Shagawa Lake was chosen by the Federal Water Pollution Control Administration as a suitable candidate for a lake restoration demonstration project. It was desired to demonstrate the restoration of a eutrophic lake which owed its problems to the input of domestic secondary sewage. The method to be tested was advanced wastewater treatment for phosphorus removal as a means to drastically reduce the phosphorus loading of the lake and concomitantly its productivity.

Prior to the decision in 1971 to build the tertiary sewage treatment facility at Ely, several years of data were collected to characterize the limnology of Shagawa Lake. Also, a pilot tertiary treatment plant was built in 1967 as a part of a special series of experiments designed to determine if this approach would work. Included in that preliminary work was a number of *in situ* algal assays. Three of these assays included the measurement of algal photosynthesis and this paper will discuss the results of those experiments as they pertain to the use of *in situ* algal assays.

METHODS

The studies were conducted in basins floating in Shagawa Lake. Three large basins holding 150,000 gallons each were constructed using neoprene impregnated nylon. They measured 12.2m x 12.2m at the surface, 1.5m x 1.5m at the bottom and were 6.1m deep. The bottoms of the basins were sealed to prevent any exchange activity while at the surface the basins were entirely open to the atmosphere. Buoyant support was provided by a tubular aluminum float system. In addition, five small basins capable of holding 80 gallons each were constructed using 100-gallon capacity plastic bags supported by styrofoam collars.

Various mixtures of Shagawa Lake water, Burntside River water and treated sewage effluent were placed in the basins along with an inoculum of the current Shagawa Lake plankton. The three large basins were filled simultaneously with lake water drawn from a point source to ensure all basins received an identical inoculum of plankton; additives were metered-in mechanically throughout the filling process. The small basins were filled manually. Two levels of tertiary treatment were tested: one designed specifically to remove phosphorus (99+%) by chemical coagulation (alum) and filtration (i.e., the CF-system), and a more elaborate scheme which incorporated a column of activated charcoal, and cation and anion exchangers (i.e., the AE-system, see Figure 1) capable of removing over 90% of all the nutrients in Ely's secondary wastewater (Brice and Powers, 1969). The small basins were sampled using a long plastic tube to remove a vertically integrated

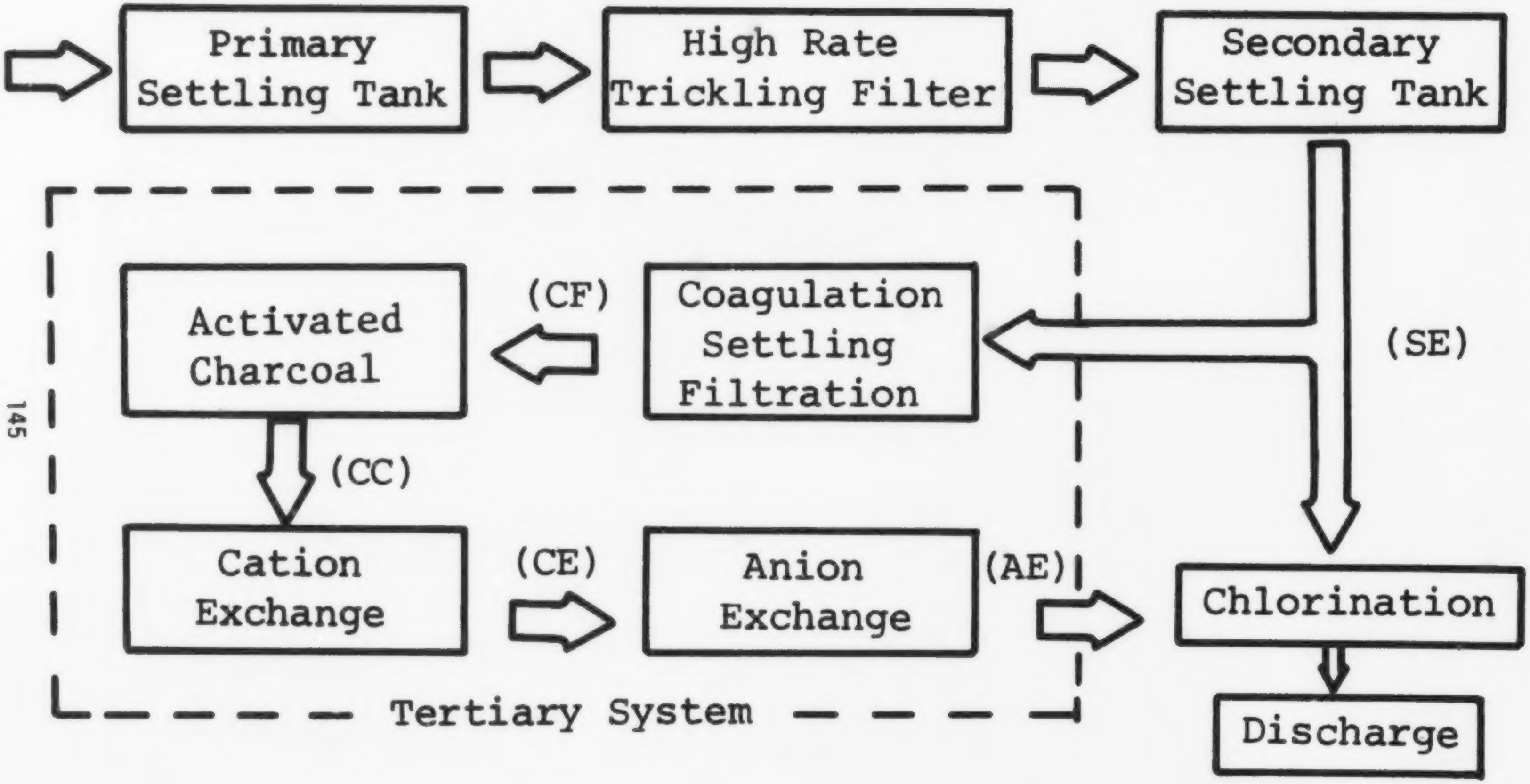

Primary Settling Tank
High Rate Trickling Filter
Secondary Settling Tank
(SE)
Coagulation Settling Filtration
(CF)
Activated Charcoal
(CC)
Cation Exchange
(CE)
Anion Exchange
(AE)
Chlorination
Discharge
Tertiary System

FIGURE 1. Sewage treatment facility block diagram

sample. The large basins were sampled at four depths in the center and a composite prepared for all analyses.

Chlorophyll a was determined in duplicate by the method of UNESCO (1966) correcting for phaeopigments (Lorenzen, 1967). Primary productivity was measured in duplicate (small basins) or triplicate (large basins) using the light- and dark-bottle oxygen method (Strickland, 1960) with a six hour *in situ* incubation period.

Each day that productivity was measured in the basins the rates of gross photosynthesis observed were compared statistically. The day's data were arranged using a one-way classification scheme (according to sewage type), an analysis of variance was performed and linear comparisons made between selected pairs of treatment means using the method of Scheffe' (Snedecor and Cochran, 1967; Guenther, 1964). Rate differences were declared significant at the 0.05 probability level (i.e. where a = 0.05).

The ratio of photosynthesis to respiration (P/R) was used to quantify the relationship between autotrophy and heterotrophy in the basin plankton and was calculated as follows:

$$P/R = \frac{\text{daily gross photosynthesis (mg C/m}^3\text{/day)}}{\text{24-hour community respiration (mg C/m}^3\text{/day)}}$$

RESULTS

The Large Basin Experiments

The objectives of these experiments were to evaluate the effectiveness of the pilot tertiary plant in reducing the capacity of Ely's secondary sewage effluent to stimulate phytoplankton primary production and to observe the effect of different effluent mixtures on the specific photosynthesis rates of Shagawa Lake algae.

Experiment 1

In 1969 the filling of the basins began June 18 and sampling was initiated June 20 when the basins were full. Planktonic photosynthesis was measured every other day until the experiment was terminated July 3. Lake water was mixed with treated sewage effluent in the ratio of 95 parts Shagawa Lake water to 5 parts effluent. Table 1 shows the composition of the mixtures in the large basins.

Table 1. Composition of the large basins in Experiment 1

Basin	% Effluent			%Shagawa Lake Water	
	SE	CF	AE	Filtered	Unfiltered
Secondary	5	-	-	-	95
CF-tertiary	-	5	-	-	95
Control	-	-	-	5	95

A 5% enrichment was first tested because preliminary experiments (Megard, 1969) had indicated this low a concentration could cause a measurable response and, indeed, it was sufficient to cause a significant increase in phytoplankton primary production.

Figure 2A shows that chlorophyll a was most abundant in the basins which received sewage additions. A maximum concentration of 46 mg/m^3 occurred after 9 days in the basin that contained 5% secondary effluent. A peak of 28 mg/m^3 was reached after 7 days in the basin that contained CF-tertiary effluent. In contrast, chlorophyll a concentrations in the control basin remained fairly stable in the range of 13-20 mg/m^3.

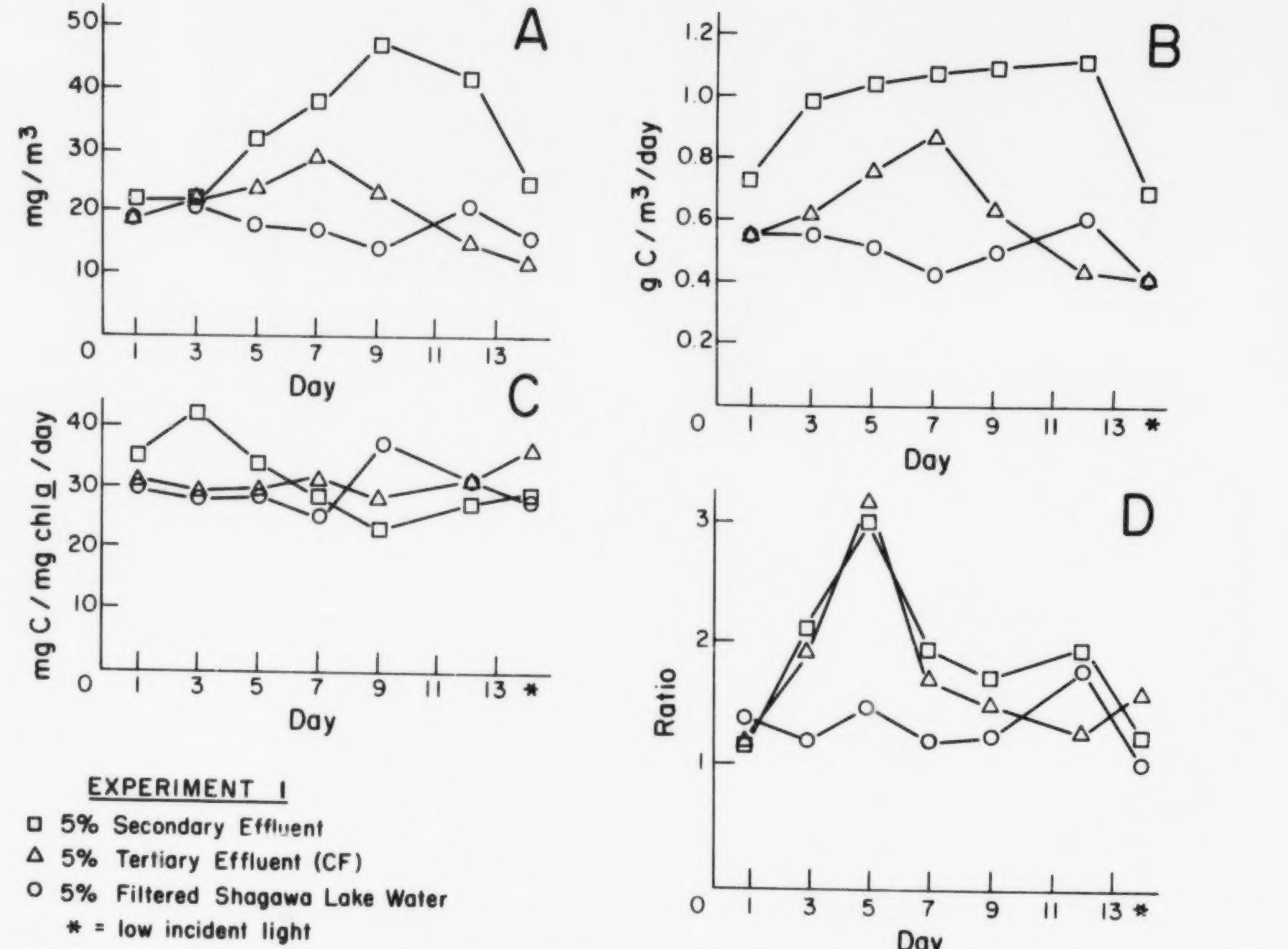


FIGURE 2. A. Chlorophyll a concentrations, B. Gross Photosynthesis, C. Specific gross photosynthesis, D. Ratio of daily gross photosynthesis to 24-hour community respiration (P/R)

Rates of gross photosynthesis (Figure 2B) were significantly greater in the basins containing sewage effluents and rates in the basin with secondary effluent were consistently higher than those in the basin with CF-tertiary effluent.

Figure 2C shows that specific gross photosynthesis (mg C/mg chl a/day) was significantly higher in the basin containing secondary effluent during the first 5 days of experiment 1 while values in the tertiary and control basins were quite similar. After day 5 there was no apparent treatment effect on this parameter.

The P/R ratios plotted in Figure 2D indicate a higher degree of autotrophy was present in the basins receiving sewage effluents. The mean ratio in the secondary basin (1.9) was only slightly greater than in the CF-tertiary basin (1.7). The mean ratio in the control basin was 1.3.

Experiment 2

The filling of the basins began July 15, 1969 and sampling was initiated July 16. Planktonic photosynthesis was measured every other day until the experiment was terminated July 29. Table 2 shows the composition of the mixtures in the basins. Lake water was mixed with treated sewage effluent in the ratio of 90 parts lake water to 10 parts effluent.

Table 2. Composition of the large basins in Experiment 2

Basin	% Effluent			% Shagawa Lake Water	
	SE	CF	AE	Filtered	Unfiltered
Secondary	10	-	-	-	90
CF-tertiary	-	10	-	-	90
Control	-	-	-	10	90

This 10% mixture was used to further test the effectiveness of the tertiary treatment system to reduce the capacity of secondary effluent to stimulate algal growth.

As in experiment 1 there was a treatment effect readily apparent in the photosynthesis data (Figure 3A) while the cholorphyll a data (Figure 3B) showed a lag in response.

The CF-tertiary treatment of the secondary effluent severely altered the capacity of the secondary sewage to stimulate planktonic primary productivity, and the mean rate of daily gross photosynthesis in the CF-tertiary basin was 85% less than the rate in the secondary basin, and 59% less than the mean rate in the control basin. There is reasonable doubt, which will be discussed later, that this was accomplished solely by the removal of certain nutrients from the secondary sewage. Chlorophyll a and gross photosynthesis all declined in a similar manner in both the control basin and CF-tertiary basin, but the numerical values for these parameters were significantly lower in the CF-tertiary basin in which there was also an unusually low balance between autotrophy and heterotrophy as indicated by the P/R ratios in Figure 3C.

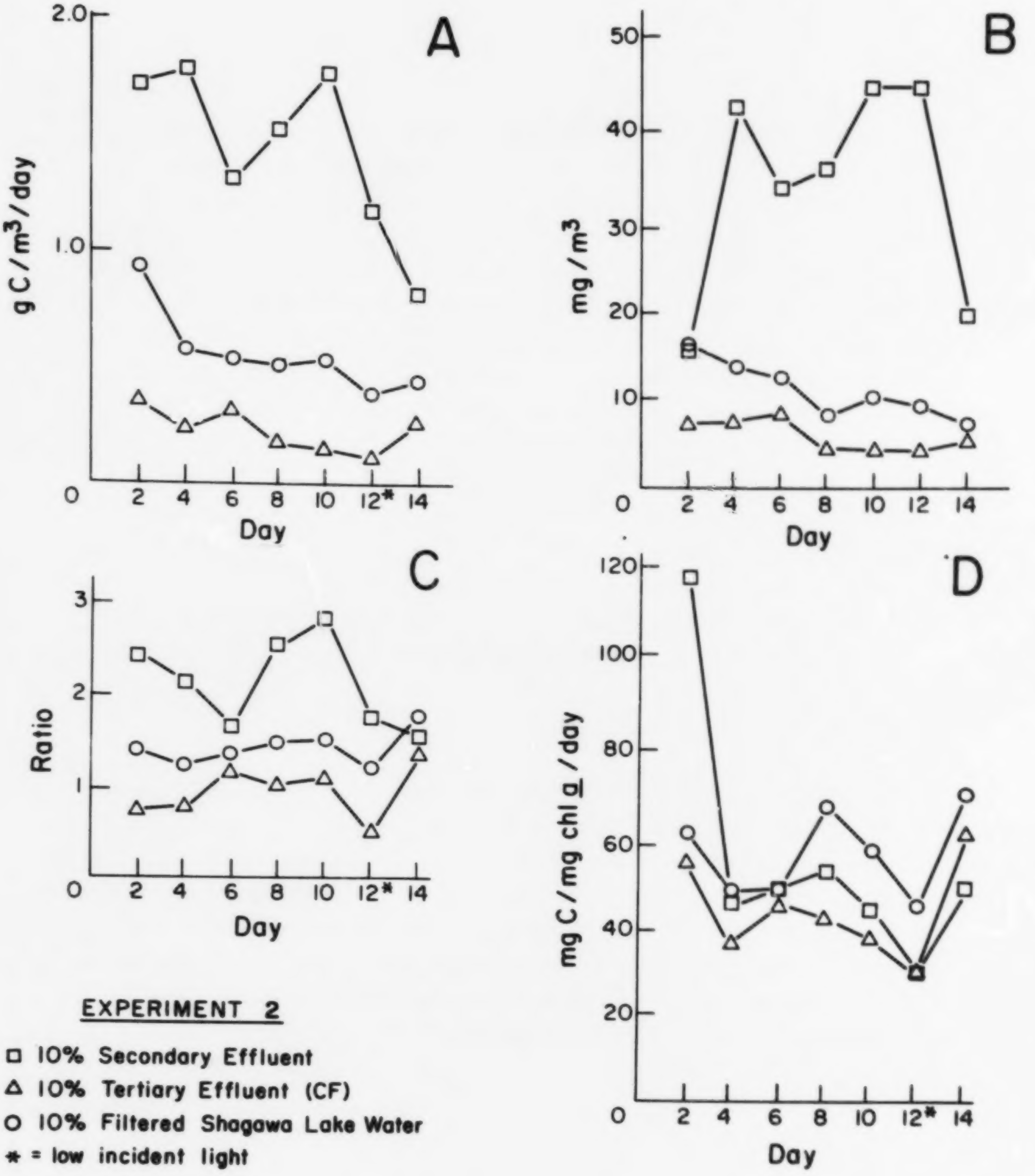


FIGURE 3.

A. Gross photosynthesis,

B. Chloropyll a concentrations,

C. Ratio of daily gross photosynthesis to 24-hour community respiration (P/R),

D. Specific gross photosynthesis

Specific rates of gross photosynthesis in the secondary basin were in general less than those in the control basin except for day 2 (Figure 3D). This was probably related to a density factor to be discussed later. While the mean specific rate of gross photosynthesis in the CF-tertiary basin (43 mg C/mg chl a/day) was 20% lower than the mean rates in the secondary and control basins (54 and 55 mg C/mg chl a/ day, respectively), it was still indicative of the presence of phytoplankton in reasonably good physiological condition, judging from *in situ* photosynthesis experiments with lake phytoplankton (Megard, 1969 and Megard and Smith, 1973). Thus it would appear that there was a factor in the CF-tertiary effluent that proved detrimental only to a discrete portion of the phytoplankton early in the experiment (i.e. prior to the first sampling). It may have been a normal constituent of the effluent that became disruptive at the 10% level of enrichment, or a transient material which entered the tertiary treatment system. It remains unidentified.

The Small Basin Experiment

The purpose of this experiment was to assess the impact that different tertiary effluents might have on the future productivity of Shagawa Lake. To achieve this some alterations were necessary in the experimental design. The most significant change was the use of Burntside River water as a base medium. Because of the unfeasibility of transporting the volume of Burntside River water necessary for filling the 150,000-gallon basins, the experiment was conducted using 80-gallon basins. Table 3 defines the composition of the basins.

Table 3. Composition of the small basins

Basin	% Effluent			% Shagawa Lake Water		Burntside River Water (1%)
	SE	CF	AE	Filtered	Unfiltered	
Secondary	5	-	-	-	1	94
CF-tertiary	-	5	-	-	1	94
AE-tertiary	-	-	5	-	1	94
Burntside R.	-	-	-	-	1	99
Shagawa Lake	-	-	-	99	1	-

All basins received 1 part Shagawa Lake water to provide a plankton inoculum; three basins received 94 parts Burntside River water plus 5 parts of a sewage effluent (SE, CF or AE); a fourth basin received 99 parts Burntside River water to serve as a control and the fifth basin received 99 parts of filtered Shagawa Lake water. The latter basin was included to test the assumption of the experimental design that the basin containing Burntside River water plus secondary sewage was, in fact, a model of Shagawa Lake. If true, similar responses would be anticipated in these two basins.

The basins were filled on August 5, 1969, and sampled immediately. Planktonic photosynthesis was measured every other day until the experiment was terminated on August 13.

In general the biological activity in the five 80-gallon basins followed two distinct patterns, one common to the Shagawa Lake basin and its experimental model, the secondary basin, and the other common to the

AE-tertiary, CF-tertiary and control basins. In the former group the parameters were indicative of a rapidly growing algal population; that is, the rates of chlorophyll a synthesis (Figure 4A), gross photosynthesis (Figure 4B), and their P/R ratios (Figure 4C) were all high relative to the other basins.

Regarding primary productivity there was no statistically significant difference ($p = 0.05$) between the secondary basin and the Shagawa Lake basin after day 3; mean daily gross photosynthesis rates in the secondary (0.82 g C/m^3/day) and Shagawa Lake (0.83 g C/m^3/day) basins were nearly identical and three times as great as the mean rate in the control basin (0.26 g C/m^3/day). There was no apparent stimulation of productivity in the CF- or AE-tertiary basins (Scheffe's test, 5% level of significance). Phytoplankton productivity in the CF-tertiary basin after day 3 was 65-74% less than in the secondary basin while it was 75-82% less in the AE-tertiary basin.

Chlorophyll a data corroborated the productivity data for all basins. It did appear from chlorophyll a and gross photosynthesis data (cf. Figures 4A and 4B) that a negative response was occurring in the AE-tertiary basin after day 5, but this could not be confirmed statistically.

Specific gross photosynthesis rates (Figure 4D) in general increased through day 7. The secondary basin did exhibit significantly higher rates on two separate days which resulted in its mean rate (39 mg C/mg chl/day) being 30% greater than the means of the other four basins which were essentially identical (i.e. 30-32 mg C/mg chl/day, see Table 6).

In general the P/R ratios followed patterns similar to those in the large basins in that the ratios achieved their greatest values in the basins

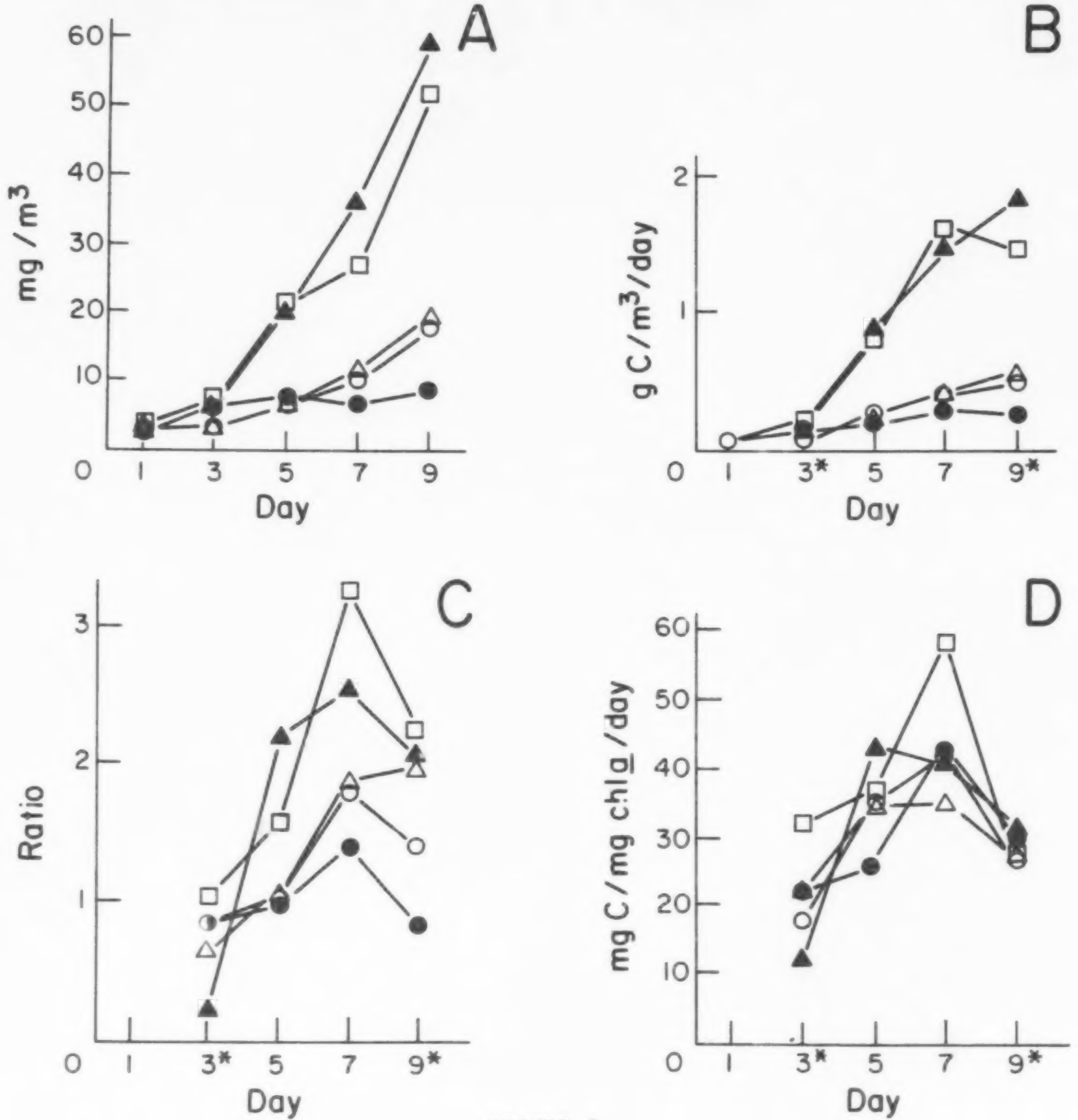


FIGURE 4.

MEDIUM BASIN EXPERIMENT

□ 5% Secondary Effluent
○ 5% Tertiary Effluent (CF)
● 5% Tertiary Effluent (AE)
△ 99% Burntside River Water
▲ 99% Filtered Shagawa Lake Water
* = low incident light

A. Chlorophyll a concentrations,
B. Gross photosynthesis,
C. Ratio of daily gross photosynthesis to 24-hour community respiration (P/R),
D. Specific Gross photosynthesis

showing the highest primary productivity. The mean P/R ratios in the secondary and Shagawa Lake basins were, respectively, 43% and 29% greater than in the control basin. The P/R ratios in the CF-tertiary basin were very similar to those in the control, while the ratios in the AE-tertiary basin began to lag after day 5 correlating with its chlorophyll decline, indicative of a waning phytoplankton population.

DISCUSSION

The Large Basin Experiments

Daily Gross Photosynthesis---

Secondary sewage effluent from the Ely municipal wastewater treatment plant exhibited a definite capacity to stimulate phytoplankton primary production. Two *in situ* large-basin experiments were conducted and in each phytoplankton productivity was statistically the greatest, by a highly significant margin (1% level of significance), in the basin receiving secondary sewage effluent. On the basis of mean gross photosynthesis rates the stimulation of productivity in the basin containing 10% secondary was 1.9 times that observed in the basin with 5% secondary (Table 4).

Table 4. A comparison of mean gross photosynthesis rates in experiments 1 and 2.

Experiment	% Eff.	Basin				
		C	CF	SE	CF	SE
		g C/m^3/day			% Stimulation	
1	5	0.50	0.59	0.94	18	88
2	10	0.58	0.24	1.57	-	171

The effect of CF-tertiary treatment was to reduce the capacity of secondary wastewater to stimulate phytoplankton production, but it also appeared to introduce a factor deleterious to some algae where an effluent concentration of 10 percent was used.

In experiment 1 (5% mixture) there was stimulation of productivity in the CF basin but on the basis of mean gross photosynthesis rates it was 70 percent less than in the secondary basin (Table 4). In experiment 2 (10% mixture) mean gross photosynthesis in the CF-basin was 59% less than in the control basin. This reflected a substantial loss of chlorophyll a in the CF-basin that occurred during the filling process and was apparent in the first day's samples (Figure 3B). Since the chlorophyll a concentration in the composition water (i.e. Shagawa Lake water) was monitored hourly it is known that the low chlorophyll level in the CF-basin was due to a loss in that basin as opposed to gains realized in the control and secondary basins. The concentrations of phaeopigments in all three basins were nearly identical at the start of the experiment suggesting the chlorophyll was lost through sedimentation and not cellular lysis.

The idea of an inhibitory factor in the CF-effluent is important enough that some reference should be made to four experiments not included in this paper. In August, 1969, a 3% mixture of CF-tertiary effluent stimulated phytoplankton productivity while mixtures containing 10% and 20% CF-effluent exhibited the inhibitory effect described above (Smith, 1973). In September, 1969, an additional experiment was conducted using a 10% mixture of CF-effluent but in this case no deleterious effect was observed (Brice, unpublished data). These results indicated the inhibitory factor was either of a transitory nature, being absent by September, or it was to some degree selective towards the kind of algae it acted upon.

Further investigation of the phenomenon was not pursued because of time and manpower limitations and the fact that the concentration necessary to bring about the effect was five-to-ten times greater than the lake would experience under actual full-scale loading conditions. However, the results do emphasize that investigators must consider possible toxic factors when engaging in experimental work with treated domestic wastewater effluents, a fact that has been documented elsewhere as well (Middlebrooks et al., 1971; Dunstan and Menzel, 1971).

P/R Ratios---

The P/R ratio serves to quantify the relationship between production and consumption in an ecosystem (Odum, 1961) and was used here to determine the relative level of autotrophy (vs. heterotrophy) in each basin.

The general effect of sewage enrichment in the large basins was to enhance autotrophy. The secondary basin consistently produced the highest mean P/R ratios in each experiment (Table 5). Examining the data it was apparent that where productivity was stimulated and the levels of both photosynthesis and respiration (Smith, 1973) increased along with biomass, the increase in photosynthetic activity was greater. This agrees with the findings of Ryther (1954) who demonstrated that the P/R ratios of *Dunaliella euchlora* cultures were a function of the nutrient status of the algae with photosynthesis rates declining more rapidly than respiration rates at the onset of nutrient deficiency.

Table 5. Photosynthesis/respiration (P/R) ratios observed in two large basin experiments. Mean (range).

Experiment	% Effluent	Basin		
		Control	Tertiary	Secondary
1	5	1.3(1.0-1.8)	1.7(1.2-3.1)	1.9(1.2-3.0)
2	10	1.4(1.2-1.8)	1.0(0.5-1.4)	2.2(1.6-2.9)

The situation in the CF-tertiary basin was inconsistent. Photosynthesis/respiration ratios were enhanced in experiment 1 (5% mixture) but they were distinctly depressed in experiment 2 (10% mixture). Inasmuch as 1) the P/R ratios in the secondary and CF-tertiary basins of experiment 1 were very similar and 2) the P/R ratios in the control basins of experiments 1 and 2 were basically identical, it is evident that the community in the CF-basin in experiment 2 was in a stressed situation relative to the CF-effluent.

Specific Gross Photosynthesis---

While P/R ratios can be indicative of the health of an algal population, they are at best conservative estimates where a natural population is concerned due to the influence of heterotrophic respiration. A more common reference is specific photosynthesis, here related to chlorophyll a (i.e. mg C fixed/mg chl a/day). While this parameter, too, is not entirely definitive, it focuses on the photosynthetic system and has been demonstrated repeatedly to be a good general index of the physiological condition of natural algal populations and artificial cultures (Hepher, 1962; Fogg, 1966; Glooschenko and Curl, 1971).

In the large basin experiments the effect of secondary effluent was to cause an initial stimulation of specific photosynthesis which later waned, while the effect of CF-tertiary treatment was to remove any capacity to stimulate.

It is not certain what specific factors were responsible for the observed declines in the secondary basins. There is evidence from both basin and lake studies that suggests it was related in some manner to the density of the algae. In experiments 1 and 2 the rapid decline in specific photosynthesis in the secondary basins coincided with a moderate increase in chlorophyll a concentration. With respect to Shagawa

Lake, the data in Figure 5 show that mean specific photosynthesis, which had been increasing throughout the summer, exhibited a substantial decline in mid-August when the density of the late summer blue-green algal boom jumped from 28 mg/m^3 of chlorophyll _a_ to 67 mg/m^3 at the onset of an exponential growth phase.

Specific photosynthesis has been found to vary considerably in some lakes (Talling, 1957; Megard, 1970; and Elster, 1965) and very little in others (Talling, 1965). There are many factors which can influence specific photosynthesis rates such as water temperature, the nutritional status and photic history of the algae (Talling, 1966). The importance of plankton density has also been discussed in a number of papers. Some have found it to be a significant factor (Wright, 1960; Elster, 1965) while others have concluded it was not (Megard, 1970; Talling, 1965). Thus it is apparent that the manner in which the element of plankton concentration functions must be dependent on other factors involved.

Generally speaking, the mean rates of specific photosynthesis observed in each experiment (Table 6) did not differ largely from one another (i.e. ± 7-22%).

Table 6. Mean rates of specific gross photosynthesis (mg C/mg chl _a_/day)

Experiment	% Eff.	Month	Basin					Shagawa Lake _in situ_
			SE	CF	AE	C	SL	
1	5	June	32	30	-	29	-	-
2	10	July	54	43	-	55	-	-
*	5	August	39	30	30	30	32	24

* Small Basin Experiment

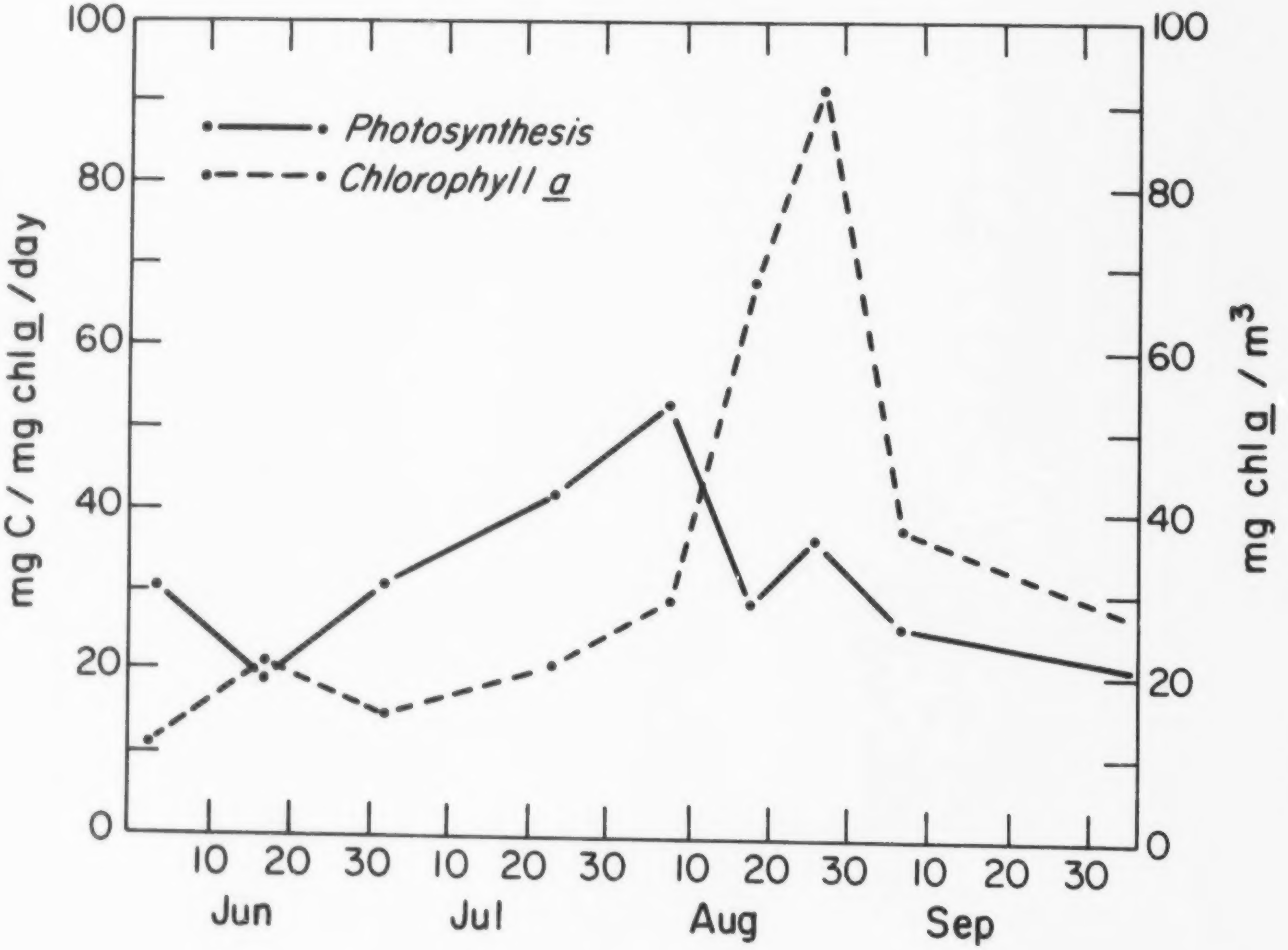


FIGURE 5. Maximum specific photosynthesis and chlorophyll a in Shagawa Lake, 1970 (Megard and Smith, 1973).

However, over the entire 1969 experimental period, the mean rates of the control basins showed a pattern of variation similar to that observed in the lake during the summer of 1970 (Megard and Smith, 1973), with elevated rates during July and lower rates in June and August (cf. Table 6 and Figure 5). Where there was stimulation of specific photosynthesis (i.e., in the secondary basins and Shagawa Lake) it appeared to be dampened by a density-related factor. In terms of chlorophyll a, a concentration of about 27 mg/m^3 appeared significant because above this level algae in the secondary basin fixed carbon at specific rates similar to those in the control basin.

The Small Basin Experiment

This experiment (5% mixture) is discussed separately from the large basin experiments because it was conducted using a different format. While four of the five basins used contained Burntside River water, a fifth was filled with filtered Shagawa Lake water. The latter basin was compared to the secondary basin to test the assumption that the mixture containing secondary effluent and Burntside River water was, in fact, an experimental model of Shagawa Lake. The AE- and CF-tertiary basins were compared to the secondary and control basins to assess the impact these tertiary effluents might have on the fertility of Shagawa Lake.

Figure 6 shows the mean gross photosynthesis rates in each basin. It is readily apparent that CF-tertiary treatment (i.e. phosphorus removal) was sufficient to thoroughly destroy the capacity of secondary effluent to enhance phytoplankton primary productivity. This is consistent with the facts that 1) the Burntside River is essentially devoid of soluble reactive phosphorus (SRP) throughout the year

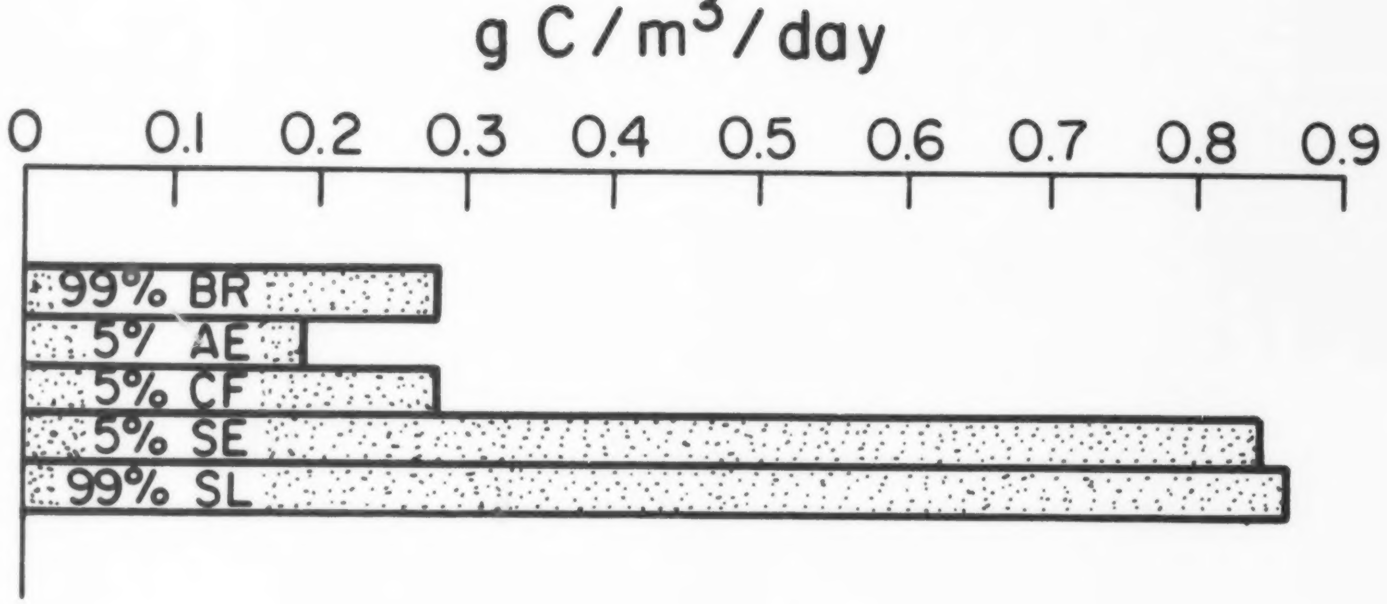


FIGURE 6. Mean rates of gross photosynthesis in the small basin experiment (BR = Burntside River, SL = Shagawa Lake, AE, CF, SE = sewage types)

(i.e. ≤ 0.001 mg/l) thus making orthophosphate a likely growth limiting factor, 2) the concentration of SRP in the CF-tertiary effluent was only 0.008 mg/l and hence contributed only 0.0004 mg/l of SRP to the assay system, an amount unlikely to cause a measurable stimulation of growth, and 3) CF-tertiary effluent would not support the growth of *Selenastrum capricornutum* without the addition of ortho-phosphate (Miller and Maloney, 1971). The results of this experiment were further supported by the *in situ* algal assays of Powers et al. (1972) which demonstrated, using phytoplankton indigenous to Shagawa Lake, that phosphorus and nitrogen together were growth limiting in Burntside River water at the times tested during the summers of 1969 and 1970.

The use of photosynthesis measurements did not offer an early insight into the responses of the subject phytoplankton as was the case in the large basin experiments. This was due in part to the sensitivity of the oxygen method which requires at least 5 μg/l of chlorophyll to permit incubation times of 6 hours or less.

The specific gross photosynthesis rates observed were of interest. While the Shagawa Lake and secondary basins supported high growth rates, specific photosynthesis was similar in all basins except for a modest stimulation in the secondary basin. This observation supports those of Talling (1966) and Megard (1970) that specific photosynthesis rates of mixed natural algal populations that are actively growing are not necessarily greater than those that are not. Thus specific photosynthesis rates are not certain guides to the nutritional status of a mixed phytoplankton population, because what is being measured is a mean weighted according to the abundance and activity of several contributing populations.

The data collected served to validate the basic assumption that the secondary basin was an experimental model of Shagawa Lake, for throughout the experiment there was generally close agreement between the parameters measured in the secondary and Shagawa Lake basins. Thus, it may be concluded that a full-scale CF-tertiary treatment system has the potential to significantly reduce the trophic status of Shagawa Lake.

The Significance of Phaeopigments

Only recently have methods been developed to permit routine analyses to be made for phaeopigments (i.e. phaeophytin and phaeophorbide) in water samples being analyzed for chlorophyll a (Yentsch and Menzel, 1963; Lorenzen, 1967).

Yentsch (1965) demonstrated the importance of correcting chlorophyll a analyses for interference from phaeopigments, particularly with deep water samples or samples near discontinuity layers. He showed that failure to apply this correction can result in sizeable underestimates of specific photosynthesis on the part of viable algae at those depths.

Phaeopigments were analyzed routinely in these experiments and it was found that they could introduce considerable error into chlorophyll a determinations in batch-tests such as are described here. In experiment 2 on day 6 the Strickland and Parson trichromatic equation indicated the chlorophyll a concentration in the secondary basin to be 45 μg/l. In fact, 44% of this value (i.e. 20 μg/l) were phaeopigments (Figure 7).

Obviously the failure to compensate for such an error would seriously affect the accuracy of any calculated values of specific photosynthesis. Table 7 gives a comparison of specific photosynthesis rates calculated from corrected and uncorrected chlorophyll a values in both the

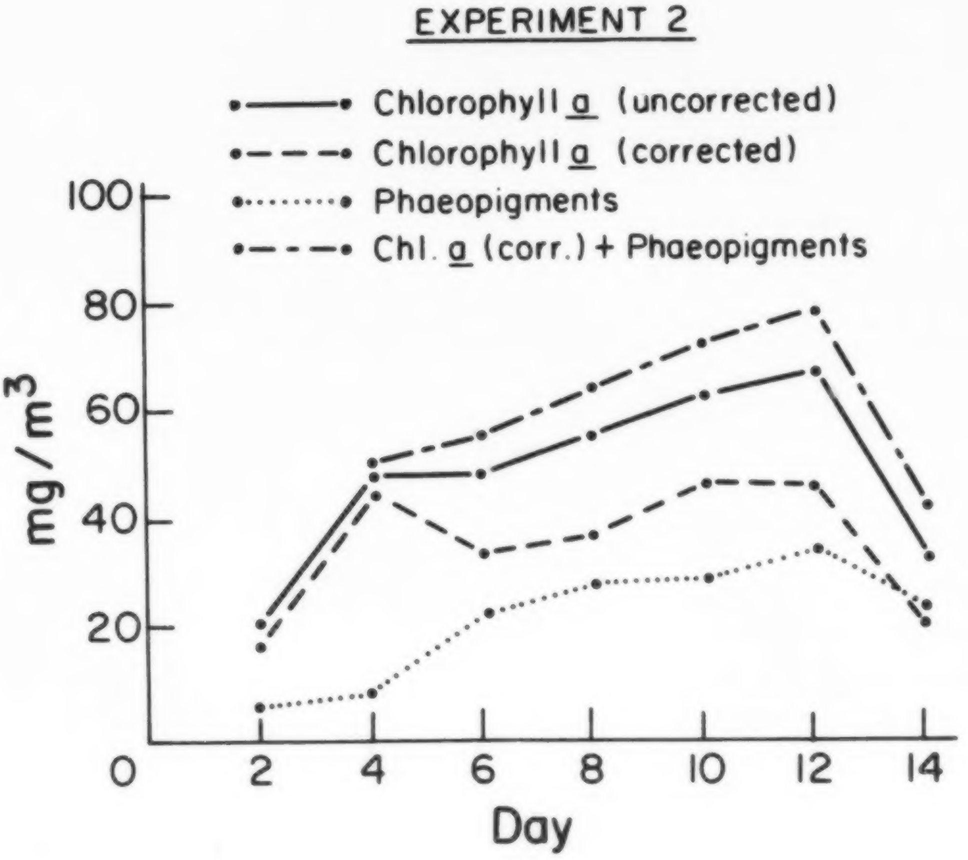


FIGURE 7. Chlorophyll a and phaeopigments in the secondary basin (10% mixture)

Table 7. A comparison of specific photosynthesis rates showing the effect of correcting chlorophyll a analyses for phaeopigment interference (experiment 2)

Day	mg C fixed/mg chlorophyll a/ day					
	Control Basin			Secondary Basin		
	Uncorrected	Corrected	% Change	Uncorrected	Corrected	% Change
2	54.9	60.2	10.6	101	116	14.9
4	37.8	46.8	23.8	39.6	44.0	11.1
6	37.8	47.0	24.3	33.7	46.7	38.6
8	46.6	65.8	41.2	34.8	50.5	45.1
10	40.8	56.0	37.2	31.5	43.4	37.8
12*	30.6	43.8	43.1	20.1	29.2	45.3
14	43.6	68.6	57.3	28.8	48.1	67.0

*Low incident light

secondary and control basin of experiment 2. The concentrations of phaeopigments in both basins were essentially the same on the initial day of sampling (Figures 7 and 8) and were typical of levels observed in Shagawa Lake during the summer growing season (Megard and Smith, unpublished data).

Thus it behooves investigators to be cautious with regard to chlorophyll a analyses. Lorenzen (1967) suggests, as a guide for screening samples, that where the ratio

$$\frac{E^{665}\ \text{(before acidification)}}{E^{665}\ \text{(after acidification)}} < 1.6$$

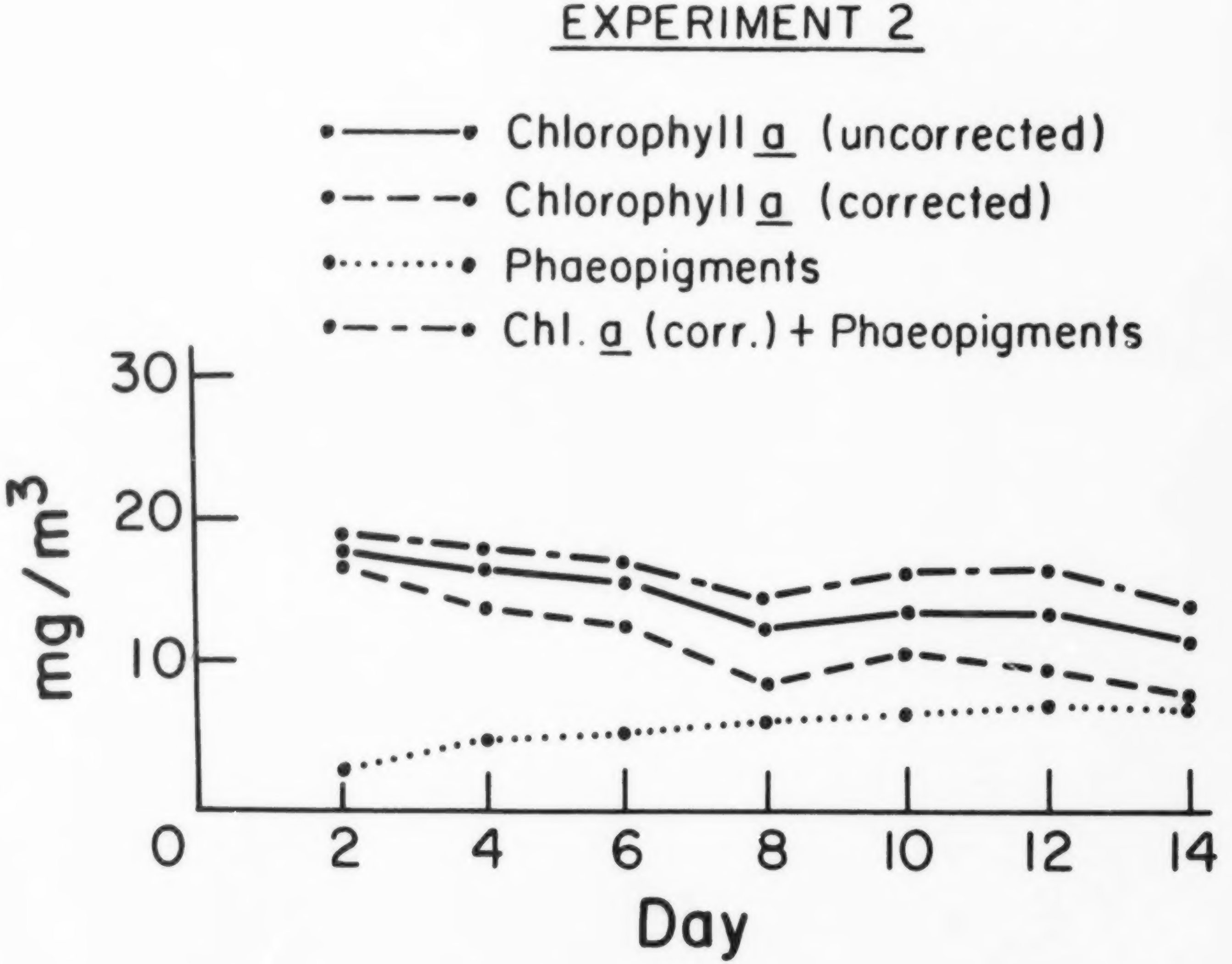


FIGURE 8. Chlorophyll *a* and phaeopigments in the control basin (10% mixture)

the presence of phaeopigments warrants quantitative consideration. The familiar empirical equations for chlorophyll a were doubtlessly generated using preparations essentially free from phaeopigments or other degradation products and thus the presence of these entities would detract from the accuracy of any chlorophyll a equation inasmuch as the degradation products of the pigment do have significant extinction coefficients in the 663 - 665 nm range. As a result equations such as those of UNESCO (1966) or Strickland and Parsons (1968) will over-estimate the presence of chlorophyll a and on the other hand they will underestimate the collective concentration of chlorophyll a and degradation products due to the extinction coefficients of the latter being smaller than those of the former in the 663 - 665 nm range (Figures 7 and 8).

CONCLUSIONS

1. Ely's secondary sewage effluent possessed a definite capacity to stimulate algal growth and specific photosynthesis; the use of CF- and AE-tertiary treatment significantly reduced that capacity.

2. CF-tertiary effluent can be detrimental to some phytoplankton in mixtures $\geq$10% wastewater.

3. Specific photosynthesis rates (i.e. assimilation ratios) are not thoroughly reliable indicators of mixed-population activity. It is recommended their use in assays as a response indicator be approached cautiously where chlorophyll a concentrations exceed 27 mg/m^3.

4. There is good probability a full-scale CF-tertiary wastewater treatment plant at Ely would result in a significant reduction in the trophic status of Shagawa Lake.

RECOMMENDATIONS: BATCH ASSAY TECHNIQUE

1. Large basins requiring many hours to fill should be sampled during the filling process to define initial responses.

2. The length of batch assays should be at least 10 days to allow the full growth potential of the test system to be realized.

3. Photosynthesis measurements can give a much earlier indication of treatment effects than chlorophyll _a_.

4. Chlorophyll _a_ measurements should consider phaeopigment interference.

ADDENDUM

A full-scale tertiary wastewater treatment facility has been constructed at Ely. It began operation January 1, 1973, and is principally a phosphorus removal plant employing lime precipitation and not alum which was used in the pilot plant studies. Algal assays by W. E. Miller and coworkers, NERC, Corvallis, using 100% tertiary effluent from the full-scale plant showed no signs of toxicity (personal communication).

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REFERENCES

Bradbury, J. P. and J. C. B. Waddington. 1973. The Impact of European Settlement on Shagawa Lake, Northeastern Minnesota, U.S.A. Limnological Research Center, University of Minnesota, Minneapolis. Contribution No. 112. 32 p.

Brice, R. M. and C. F. Powers. 1969. The Shagawa Lake, Minnesota Eutrophication Research Project. Pages 258-269 in Proceedings of the Eutrophication-Biostimulation Assessment Workshop. Pacific Northwest Water Laboratory, Corvallis, Oregon.

Dunstan, W. M. and D. W. Menzel. 1971. Continuous Cultures of Natural Populations of Phytoplankton in Dilute, Treated Sewage Effluent. Limnol. and Oceanogr. 16:623-632.

Elster, H. J. 1965. Absolute and Relative Assimilation Rates in Relationship to Phytoplankton Populations. Mem. Ist. Ital. Idrobiol. 18 Suppl:79-103.

Fogg, G. E. 1966. Algal Cultures and Phytoplankton Ecology. University of Wisconsin Press, Madison. 126 p.

Glooschenko, W. A. and H. Curl. 1971. Influence of Nutrient Enrichment on Photosynthesis and Assimilation Ratios in Natural North Pacific Phytoplankton Communities. J. Fish. Res. Bd. Canada. 28:790-793.

Guenther, W. C. 1964. Analysis of Variance. Prentice-Hall, Englewood Cliffs, N. J. 199 p.

Hepher, B. 1962. Primary Production in Fishponds and its Application to Fertilization Experiments. Limnol. and Oceanogr. 7:131-136.

Lorenzen, C. J. 1967. Determination of Chlorophyll and Phaeopigments: Spectrophotometric Equations. Limnol. and Oceanogr. 12:343-346.

Malueg, K. W., R. M. Brice, D. W. Schults and D. P. Larsen. 1973. The Shagawa Lake Project. U. S. Environmental Protection Agency, National Environmental Research Center, Corvallis, Oregon. EPA-R3-73-026.

Megard, R. O. 1969. Algae and Photosynthesis in Shagawa Lake, Minnesota. Limnological Research Center, University of Minnesota, Minneapolis. Interim Report No. 5. 20 p.

________. 1970. Lake Minnetonka: Nutrients, Nutrient Abatement, and Photosynthetic System of the Phytoplankton. Limnological Research Center, University of Minnesota, Minneapolis. Interim Report No. 7. 210 p.

Megard, R. O. and P. D. Smith. 1973. Mechanisms that Regulate Growth Rates of Phytoplankton in Shagawa Lake, Minnesota. Limnol. and Oceanogr. 19:279-296.

Middlebrooks, E. J., D. B. Porcella, E. A. Pearson, P. H. McGauhey, and G. A. Rohlich. 1971. Biostimulation and Algal Growth Kinetics of Wastewater. J. Water Poll. Control Fed. 43:454-473.

Miller, W. E. and T. E. Maloney. 1971. Effects of Secondary and Tertiary Wastewater Effluents on Algal Growth in a Lake-River System. J. Water Poll. Control Fed. 43:2361-2365.

Odum, E. P. 1961. Factors Which Regulate Primary Productivity and Heterotrophic Utilization in the Ecosystem. Pages 65-71 in Algae and Metropolitan Wastes. U. S. Dept. Health, Educ., Welfare, Public Health Service. Pub. No. SEC TR W61-3.

Powers, C. F., D. W. Schults, K. W. Malueg, R. M. Brice, and M. D. Schuldt. 1972. Algal Responses to Nutrient Additions in Natural Waters. II. Field Experiments. p. 141-156 in G. E. Likens, ed. Nutrients and Eutrophication. Am. Soc. Limnol. Oceanogr. Spec. Symp. 1.

Ryther, J. H. 1954. The Ratio of Photosynthesis to Respiration in Marine Plankton Algae and its Effect on the Measurement of Productivity. Deep Sea Res. 2:134-139.

Smith, P. D. 1973. Studies on the Influence of Sewage Effluents on Phytoplankton Productivity in Experimental Ponds. M. S. Thesis. University of Minnesota, Minneapolis. 124 p.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods. 6th ed. Iowa State University Press, Ames, Iowa. 593 p.

Strickland, J. D. H. 1960. Measuring the Production of Marine Phytoplankton. Fish. Res. Bd. Canada. Bull. 122. 172 p.

Strickland, J. D. H. and T. R. Parsons. 1968. A Practical Handbook of Sea Water Analysis. Fish. Res. Bd. Canada. Bull. 167. 311 p.

Talling, J. F. 1957. Photosynthetic Characteristics of Some Freshwater Plankton Diatoms in Relation to Underwater Radiation. New Phytologist 56:29-50.

________. 1965. The Photosynthetic Activity of Phytoplankton in East African Lakes. Int. Rev. Ges. Hydrobiol. 50:1-32.

________. 1966. Photosynthetic Behavior in Stratified and Unstratified Lake Populations of a Planktonic Diatom. J. Ecology 54:99-127.

UNESCO. 1966. Determination of Photosynthetic Pigments in Seawater. Monogr. Oceanogr. Methodol. 1. New York, N. Y. 69 p.

Wright, J. C. 1960. The Limnology of Canyon Ferry Reservoir: III. Some Observations on the Density Dependence of Photosynthesis and Its Cause. Limnol. and Oceanogr. 5:356-361.

Yentsch, C. S. 1965. The Relationship Between Chlorophyll and Photosynthetic Carbon Production with Reference to the Measurement of Decomposition Products of Chloroplastic Pigments. Mem. Ist. Ital. Idrobiol. 18 Suppl:323-346.

Yentsch, C. S. and D. W. Menzel. 1963. A Method for the Determination of Phytoplankton Chlorophyll and Phaeophytin by Fluorescence. Deep Sea Res. 10:221-231.

THE DEVELOPMENT OF A STANDARDIZED MARINE ALGAL ASSAY PROCEDURE FOR NUTRIENT ASSESSMENT

David T. Specht and William E. Miller

INTRODUCTION

The need for a means to assess the biological availability of nutrients in estuarine and marine waters is no less urgent than for freshwaters. Estuaries are particularly valuable and sensitive resources (Ketchum, 1971, Inman and Brush, 1973) for which no standardized primary producer bioassay for water quality exists. Algal bioassays have been developed to suit specific purposes, but most are narrow in design and require highly specialized personnel to operate and evaluate (Atkins, 1923; Smayda, 1970; Goldman, Tenore and Stanley, 1973; Skulberg, 1964; Glass, 1973; Dierberg, 1972; McPhee, 1961; Tarzwell, 1971; Mitchell, 1973; Tallqvist, 1973; Fitzgerald, 1972).

Inasmuch as estuaries are the "nurseries" of virtually all commercially important marine shellfish, fish, and their food organisms, it becomes vitally important to be able to accurately assess their capabilities for primary productivity and to determine and predict the impact of the addition of various nutrients and wastewaters upon that productivity (Edmondson and Edmondson, 1947; Goldman, Tenore and Stanley, 1973; Thomas, 1971; Ryther and Dunstan, 1971; Mitchell, 1973; Tallqvist, 1973(b); O'Sullivan, 1971; Apollonia, 1973; Waite and Mitchell, 1972; Preston and Wood, 1971; Ryther and Guillard, 1959; Ryther, 1954; Ketchum, 1971).

For reasons of simplicity and consistency, the protocol for the Marine Algal Assay Procedure follows closely that of the widely accepted Freshwater Algal Assay Procedure: Bottle Test (AAP) (National Eutrophication Research Program, 1971). This is particularly true of the physical facilities required, incubation conditions and methodology.

Establishment of Criteria for Bioassay Organism

Unlike freshwater systems, estuaries in particular pose the problem of interpretation of the effect of varying salinities on the effect of a given concentration of a nutrient on the biomass production of a given alga. While no algal species was thought to be unaffected by varying salinity, the most ideal candidate would be one that responded in a linear, predictable manner to such changes. A species was desired that would meet this important criterion as well as be genetically stable, reproduce primarily asexually, and not clump or tend to adhere to the walls of the flasks.

Other major problem areas to be considered were the array of factors that govern the consistency of growth response, such as light intensity, temperature, influence of pH, relative size of inoculum, and selection and modification of a standard stock medium in which intracellular nutrient carryover in the inoculum would be minimal.

A significant consideration is the ease with which algal biomass production could be measured. Consistent with the AAP, all results would be converted to equivalent dry weight, defined by standard gravimetric methods (NERP, 1971).

Selection of Candidate Test Species

Considering the established criteria, we selected candidate algal species on several bases, looking for three classes of algae. To demonstrate biostimulation, we evaluated Dunaliella tertiolecta (DUN clone) and Nannochloris atomus, two unicellular green algae. To look at toxicity or inhibition possibilities, we are considering Isochrysis lutheri, Cyclotella menenghiana, and Thalassiosira pseudonana, typical crustacean or bivalve food organisms. We also hope to look at nitrogen fixing marine blue-green algae, such as *Coccochloris elabens* or *Tichodesimium* sp. (Taylor, Lee and Bunt, 1973). These algae represent three taxonomic classes: greens, diatoms, and blue-greens. Thus far, most of the work has centered around the green flagellate, *Dunaliella tertiolecta* Butcher (DUN clone), kindly provided by Dr. John Ryther, Woods Hole Oceanographic Institution. This alga has shown the most linear response for every parameter examined so far. A distinct advantage of *Dunaliella* is that it requires no outside source of vitamins (Provasoli, 1963). Some comparison test runs of field samples have been conducted with *Thalassiosira pseudonana* Hasle and Heindal (CN clone) provided by Dr. Paul Hargraves of the University of Rhode Island, but more background work is needed on salinity-nutrient interaction. After the needed information on the diatom is assembled, more intensive efforts will be made in search of a suitable blue-green alga.

Development of Media Response Curves

The initial work with *Dunaliella* was performed using a modified version of Burkholder's Artificial Sea Water (ASW) (Burkholder, 1963). This is one of several potentially amenable media which will support algal growth at relatively low nutrient levels (Kester, Duedall, Connors, and Pytkowicz, 1967; Lyman and Fleming, 1940) (see Table 1).

Salinity, Tolerance and Salt Interaction

Although it was assumed that all algae would be affected to some degree by the salinity of the media or samples, it was desirable to find the alga with the most linear response and tolerance to manipulation of salinity, primarily between 5 °/oo (ppt) and 35 °/oo. In order to establish linearity of response, levels of salinity chosen were 5, 8, 12, 16, 20, 24, 30, and 35 °/oo (Figure 1). The initial salinity tests

TABLE 1. MARINE ALGAL ASSAY PROCEDURE SUMMARY

I. TEST ORGANISM: *Dunaliella tertiolecta* Butcher (DUN clone) available from the Eutrophication and Lake Restoration Branch, PNERL, EPA. 200 SW 35th St., Corvallis, OR 97330; or WHOI.

II. ENVIRONMENTAL CONDITIONS: In general, follow exactly the ALGAL ASSAY PROCEDURE BOTTLE TEST (NERP, EPA, August, 1971) with the following exceptions:

TEMPERATURE:	18 - 20°C
SAMPLE SIZE:	100 ml in a 500 ml Erlenmyer flask
INOCULUM:	Inoculate with 1 ml washed (w/ 20 °/oo ASW less N & P, by serial centrifugation) 5-8 day old culture at a concentration of 10,000 cells/ml (to give a final concentration in the flask of 100 cells/ml, equivalent to approximately 0.03 mg dry wt/ℓ).

III. BASAL MEDIUM: Modified Burkholder's Artificial Seawater (ASW) with NAAM levels of the following nutrients; N, P, Fe, and Na_2EDTA Use Analytical Reagent or Reagent Grade chemicals, and double glass distilled water.

Compound		grams/ℓ	grams/4ℓ
NaCl		23.48	93.92
Na_2SO_4		3.92	15.68
$NaHCO_3$		0.19	0.76
KCL		0.66	2.64
KBr		0.096	0.384
H_3BO_3		0.026	0.104
$MgCl_2 \cdot 6H_2O$		10.61	42.44
$SrCl_2 \cdot 6H_2O$		0.04	0.16
$CaCl_2 \cdot 2H_2O$		1.469	5.876
H_2O	to	1,000 ml	4,000 ml

Filter the above through prewashed 0.45 μm membrane filter.

FOR DILUTION TO VARIOUS SALINITIES: (4 liter batches)

Salinity, °/oo	ASW stock, ℓ	H_2O, ℓ (glass distilled)
35	4.0	0.0
30	3.428	0.571
24	2.742	1.257
20	2.285	1.714
16	1.828	2.171
12	1.371	2.628
8	0.914	3.085
5	0.571	3.428

For any given final salinity, mix well, adding the following NAAM levels of nutrients:

$NaNO_3$	102 mg/4ℓ batch (4.2 mg N/ℓ)
K_2HPO_4	4.176 mg/4ℓ batch (0.186 mg P/ℓ)
Na_2EDTA	1200 μg/4ℓ batch (300 μg/ℓ)

*NAAM trace metal mix (minus $FeCl_3$)
filter through 0.45 μm membrane filter,
add AFTER filtration, sterilized $FeCl_3$, 384 μg/4ℓ batch (33.05 μg Fe/ℓ).

* Add the following: 0.0928 g H_3BO_3; 0.208 g $MnCl_2 \cdot 4H_2O$; 0.016 g $ZnCl_2$; 0.714 mg $CoCl_2 \cdot 6H_2O$; 0.0107 mg $CuCl_2 \cdot 2H_2O$; 3.63 mg Na_2MoO_4 $2H_2O$; make up to 500 ml, adding 1 ml of this concentrate to each liter of media.

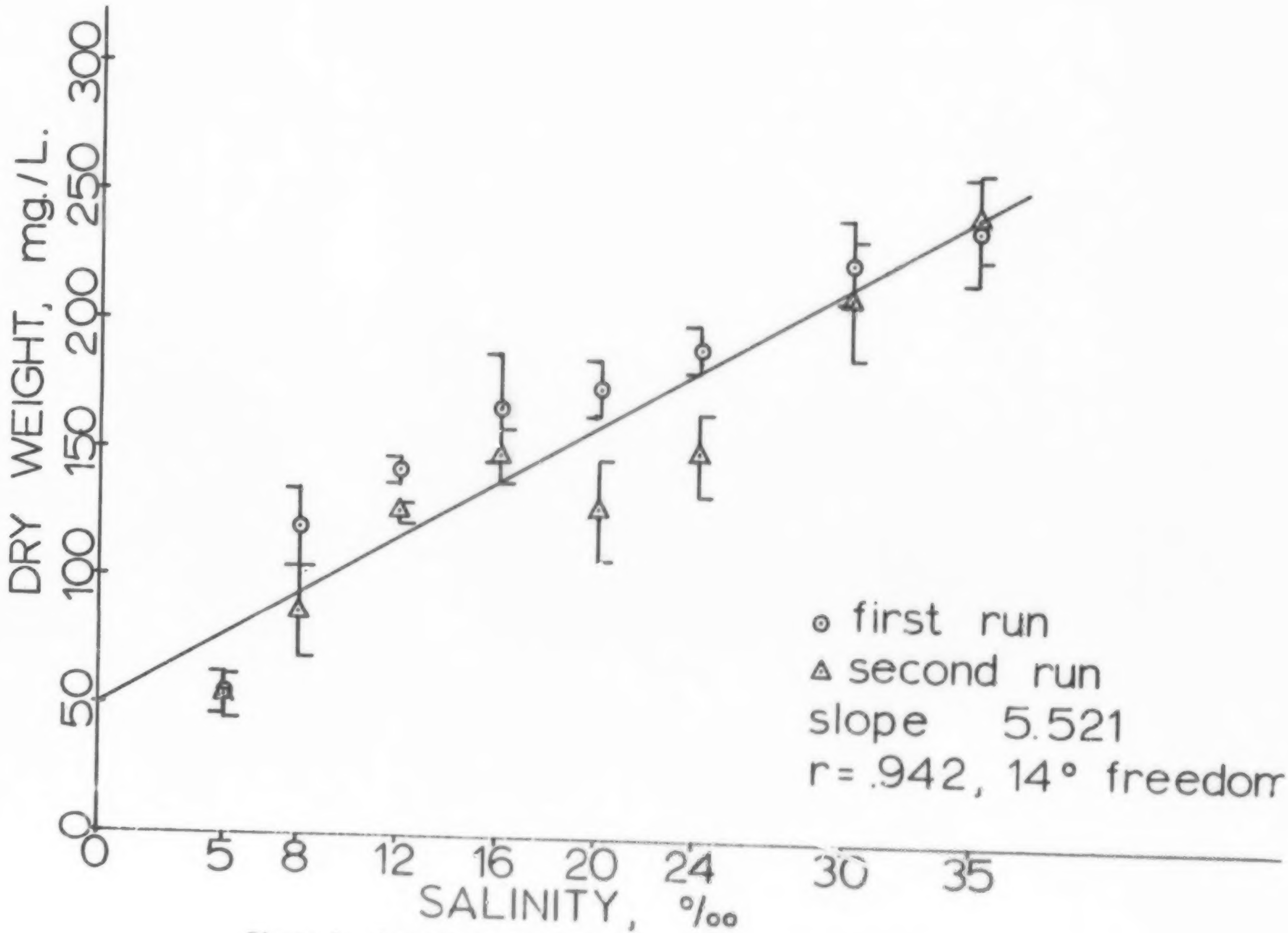


Figure 1. *Dunaliella tertiolecta*---Burkholder's ASW + NAAM nutrients

were made with rather high levels of nutrients, but later nutrient response trials showed essentially the same linearity at much lower levels. The data thus produced provided the basis for the three-dimensional plots such as Figures 2, 3, and 4, which portray response "surfaces" according to salinity-nutrient interactions. These responses appear to be predictable, within limits (Figures 2, 3, and 4).

Temperature and Light

Several trials were made at 24 ± 2°C, the standard temperature used in the freshwater Algal Assay Procedure. However, responses were both irregular and too rapid for accurate assessment; and replication was particularly poor. A suggested temperature of 18°C was found to yield satisfactory results in these same terms (McLachlan, 1960).

Light levels of 400 ft-c ± 10 percent (1300 $\mu w/cm^2$; 4300 lux)[a] proved satisfacotry for Dunaliella, and no reason was seen to change this from the freshwater Algal Assay Procedure (NERP, 1971).

The work so far with Thalassiosira has been done at 20 ± 2°C, with no shaking, and 550 ft-c ± 10 percent (1850 $\mu w/cm^2$; 5920 lux), according to data provided by Larsen (1973) in his work with this species. Good replication has been obtained under these conditions.

---

[a] The energy level output of a bank of six 48 inch "cool white" fluorescent lamps (GE 40 watt, @ 60 Hz) was approximately 1300 $\mu w/cm^2$ (range, 380-760 nm) at a distance of 26 3/4 inches, as measured with a ISCO model SRC spectroradiometer. Using the same measurement geometry, a Weston Model 756 Illumination Meter read 400 footcandles. All reflecting surfaces were matte white.

Therefore, utilizing a calibrated illumination meter with a footcandle readout, one may, by adjusting the height of the lights, achieve a known energy level output of 1300 $\mu w/cm^2$.

For further discussion of the problems of the differences in absorption of light by photosynthesizing organisms and by man's eye and their measurement, see Tyler (1973).

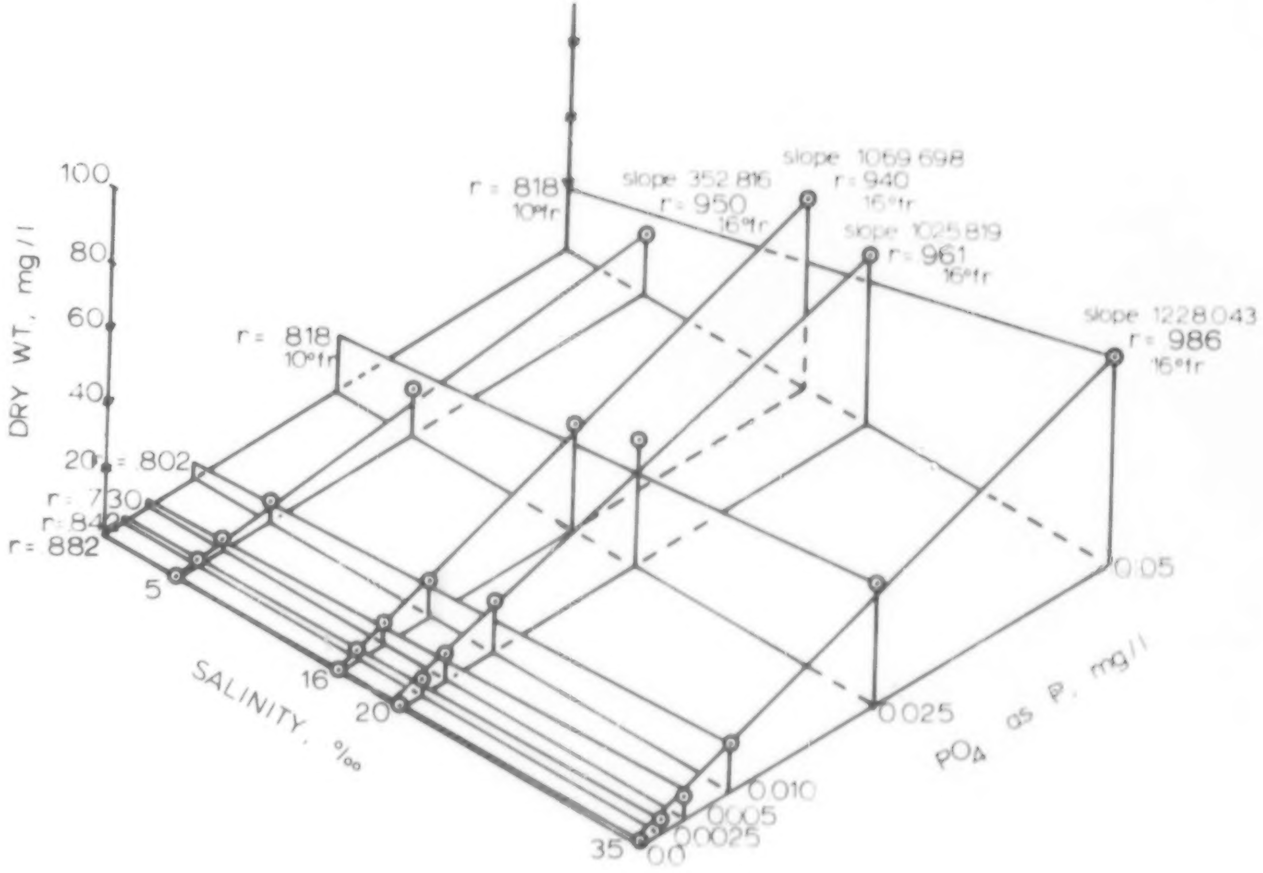


Figure 2. Growth of Dunaliella at various salinities and phosphorus concentrations in ASW. Dry weight at day 10.

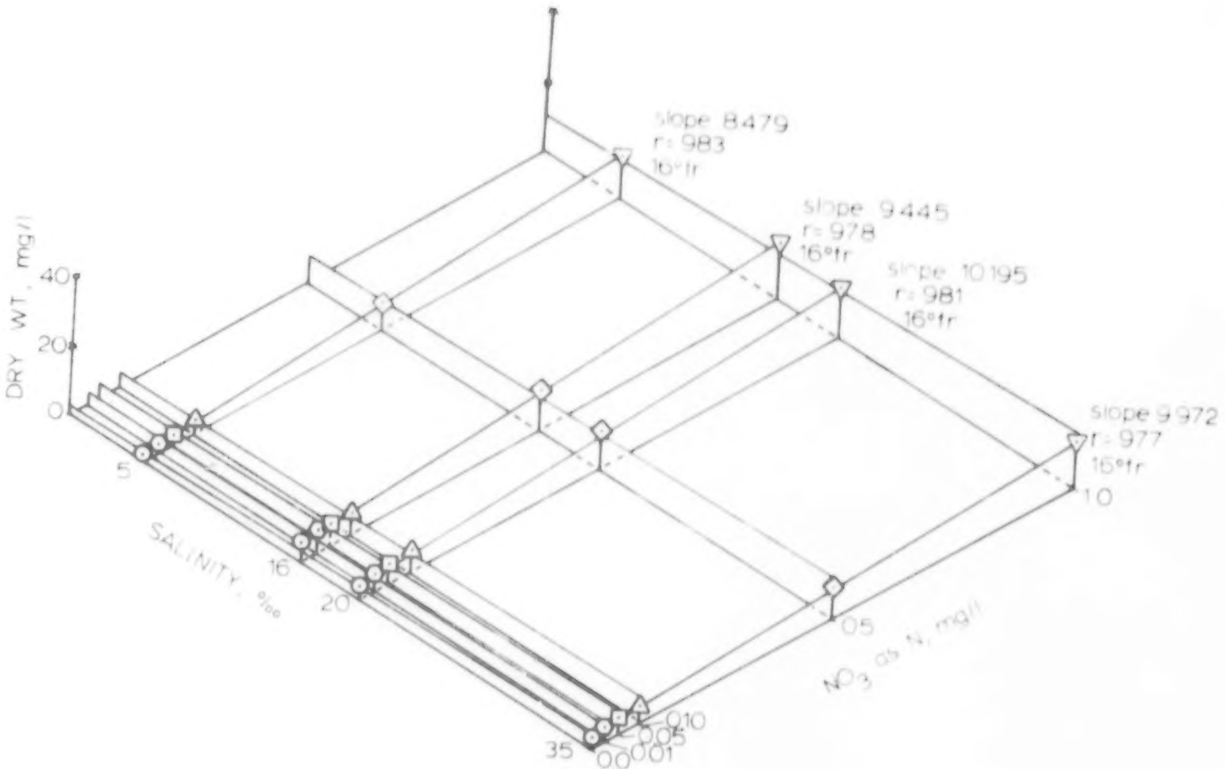


Figure 3. Growth of Dunaliella at various salinities and nitrate nitrogen concentrations in ASW. Dry weight at day 10.

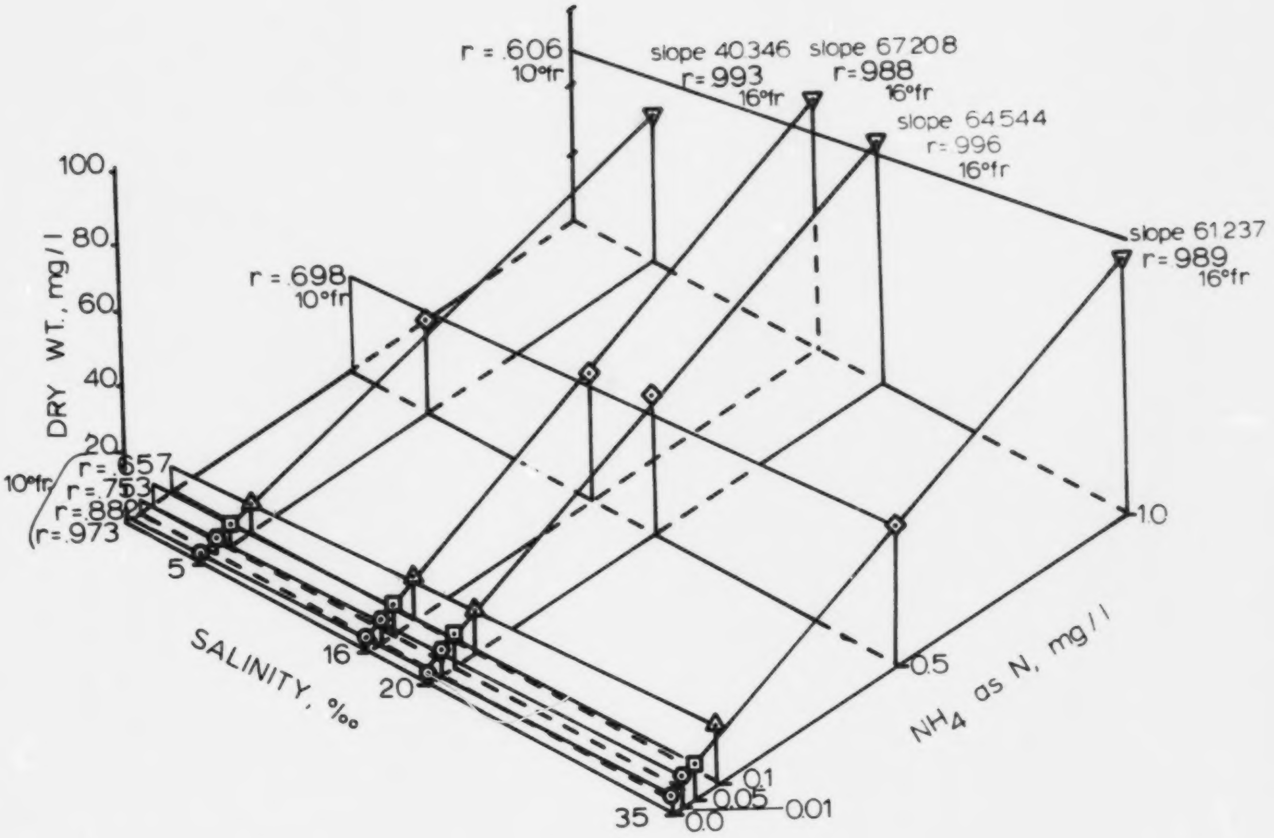


Figure 4. Growth of *Dunaliella* at various salinities and ammonia nitrogen concentrations in ASW. Dry weight at day 10.

Biomass Determination

Several methods of growth measurement, indirect or direct, may be employed. Direct cell counting, using a haemocytometer with a microscope, is relatively accurate at high population levels, but very slow. Gravimetrically determined dry weights are likewise time consuming and may be at variance with calculated dry weights if substantial bacterial growth occurs in samples, or substantial amounts of ferric-magnesium hydroxide forms in the sample. Also, accurate dry weights are difficult to obtain at low algal biomass concentrations.

In the development of this test, electronic particle counters were used to provide numerical cell counts and volumetric measurements (mean cell volume) of the samples. Samples were diluted at a ratio of 1:10 or 1:100, depending on the cell population density, with filtered isotonic saline (1% NaCl) electrolyte. Periodic cross checks for accuracy were made by haemocytometer counts and actual gravimetric dry weights. Since *Dunaliella* is substantially larger than *Selenastrum capricornutum* (AAP test species), aperture current and amplification settings used for *Dunaliella* are generally one-half that used to count *Selenastrum* in the freshwater assay procedure. Generally, the electronic particle counter was found to be the most feasible method of dealing with large numbers of samples, but may not be economically justified for casual or infrequent assays.

Nutrient Response

The initial assumption was that estuarine waters are nitrogen limited for algal growth (Ryther and Dunstan, 1971; Ryther and Guillard, 1959; Goldman, Tenore, and Stanley, 1973; Welch, 1968; Eppley, Carlucci, Holm-Hansen, Kiefer, McCarthy, and Williams, 1971; Thomas, 1970). One recent sampling survey of the Yaquina estuary showed that while water from the lower bay was predictably nitrogen limited for algal growth, water from the upper bay and its tributaries was shown to be phosphorus limited for algal growth. The Marine Algal Assay Procedure, using *Dunaliella*, can serve to define this boundary, which can change not only with the season, but with each tide, and fluctuations of tributary inflow.

With respect to this situation, the response curves developed for *Dunaliella* show that the alga will respond to concentrations at least as low as 2.5 μg P/l, 10 μg ammonia N/l, and 50 μg nitrate N/l in defined medium.

These curves were developed from additions (spikes) ranging from 2.5 to 50 μg/l in five steps for phosphorus (Figure 5), 10 to 1,000 μg in five steps for nitrate nitrogen (Figure 6) and ammonia nitrogen (Figures 7 and 8). All assays were conducted in triplicate along with controls. Nitrilotriacetic acid (NTA) was examined as a possible nitrogen source (Erickson, Maloney and Gentile, 1970; Rudd, Townsend, and Hamilton, 1973; Sturm and Payne, 1973; Mitchell, 1973), or for possible growth depressing or enhancing effects by chelation in actual estuaries field samples. There were no significant yield differences between control and spikes ranging from 50 to 1,000 mg/l NTA nitrogen (expressed as nitrogen, 7.5% N by weight).

Although ammonia nitrogen was more stimulatory than nitrate nitrogen in our ASW (Paasche, 1971), there was a growth rate difference, but no yield difference, in spiked field samples (Figures 15, 16, 18, and 19).

In Burkholder's ASW 5 ‰ to 35 ‰ salinity, *Dunaliella* produces an average of 1.076 mg dry weight per μg of phosphorus, 0.0318 mg dry weight per μg of nitrate nitrogen, and 0.0796 mg dry weight per μg of ammonia nitrogen (Table 2).

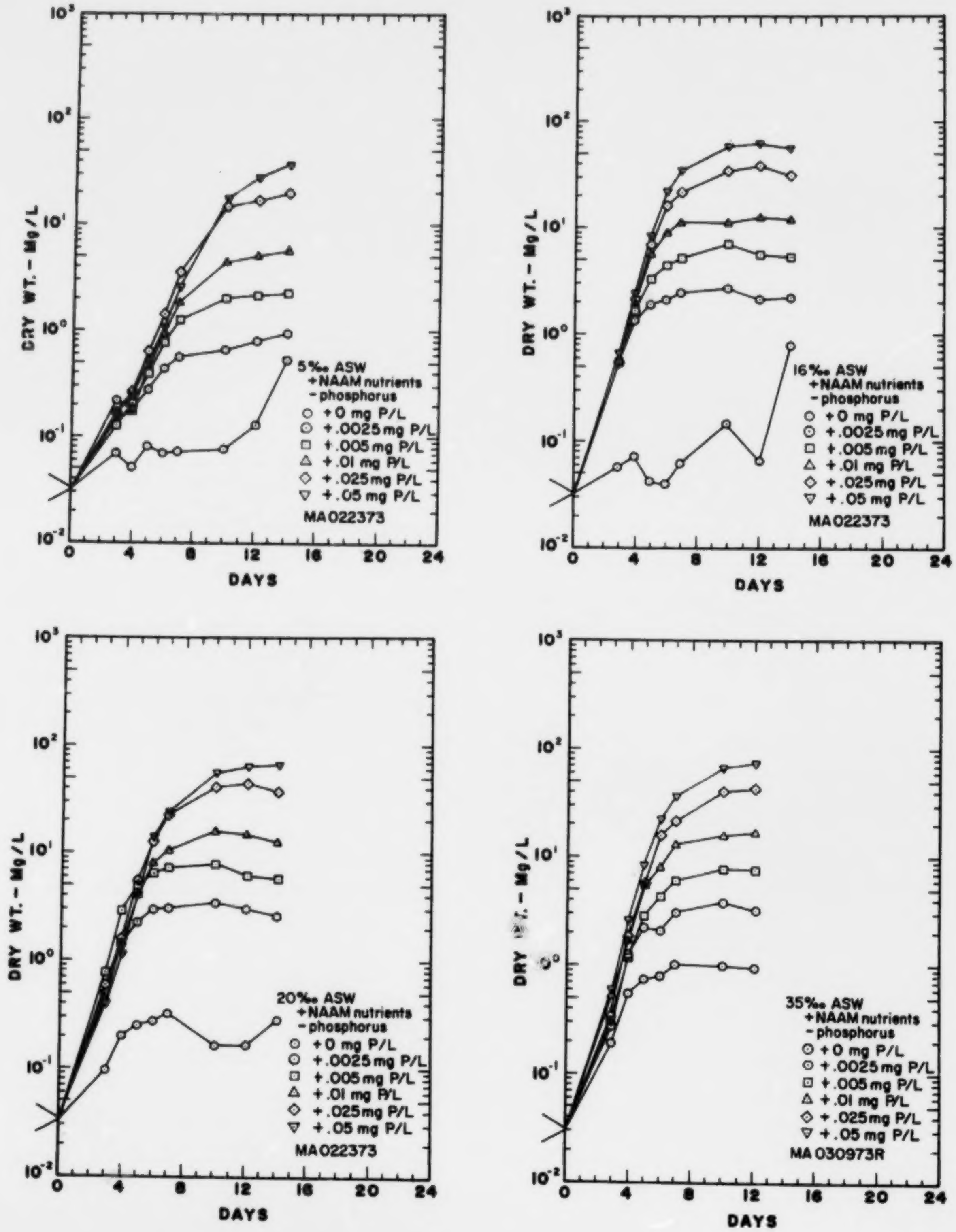


Figure 5. Growth response of *Dunaliella tertiolecta*

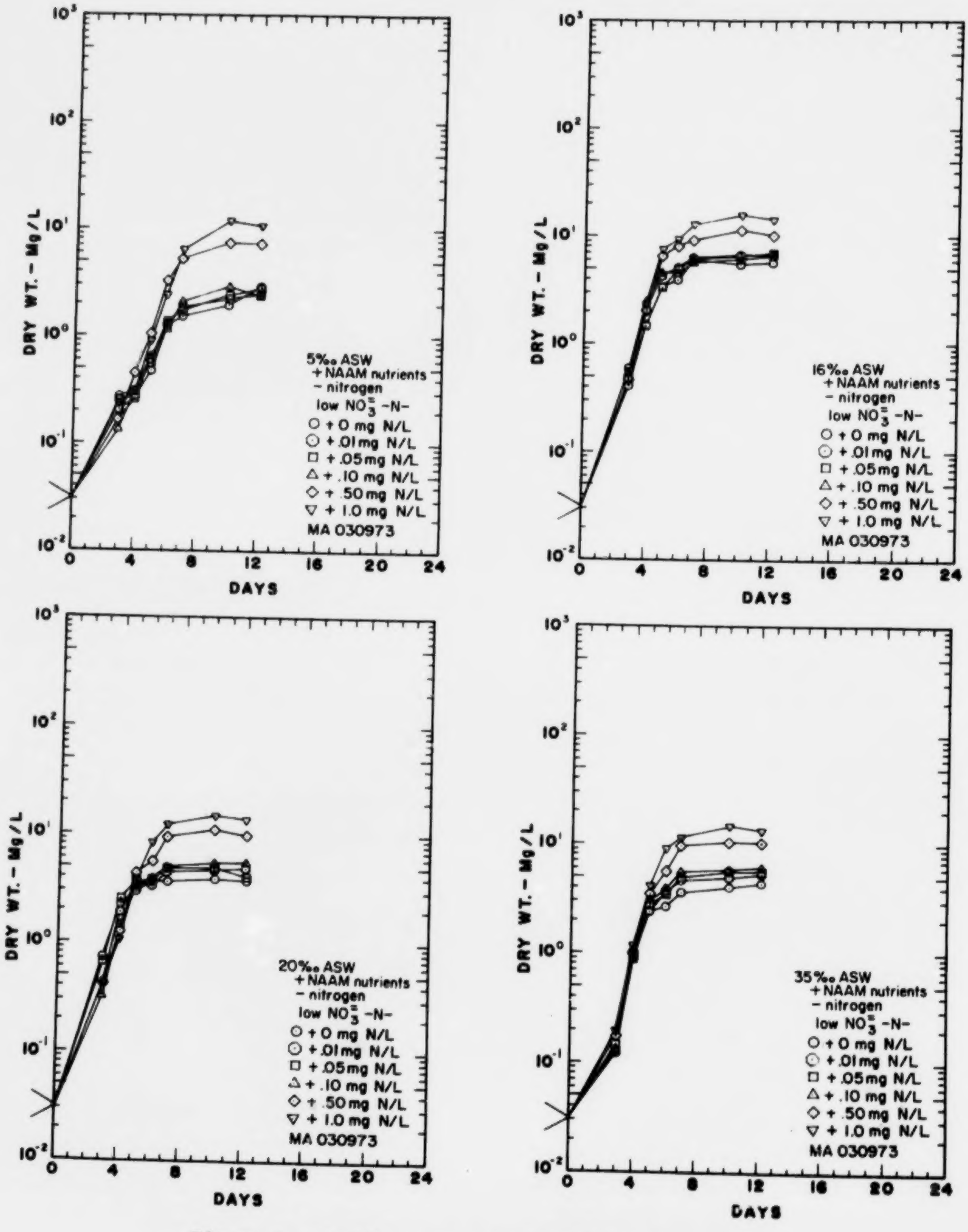


Figure 6. Growth response of *Dunaliella tertiolecta*

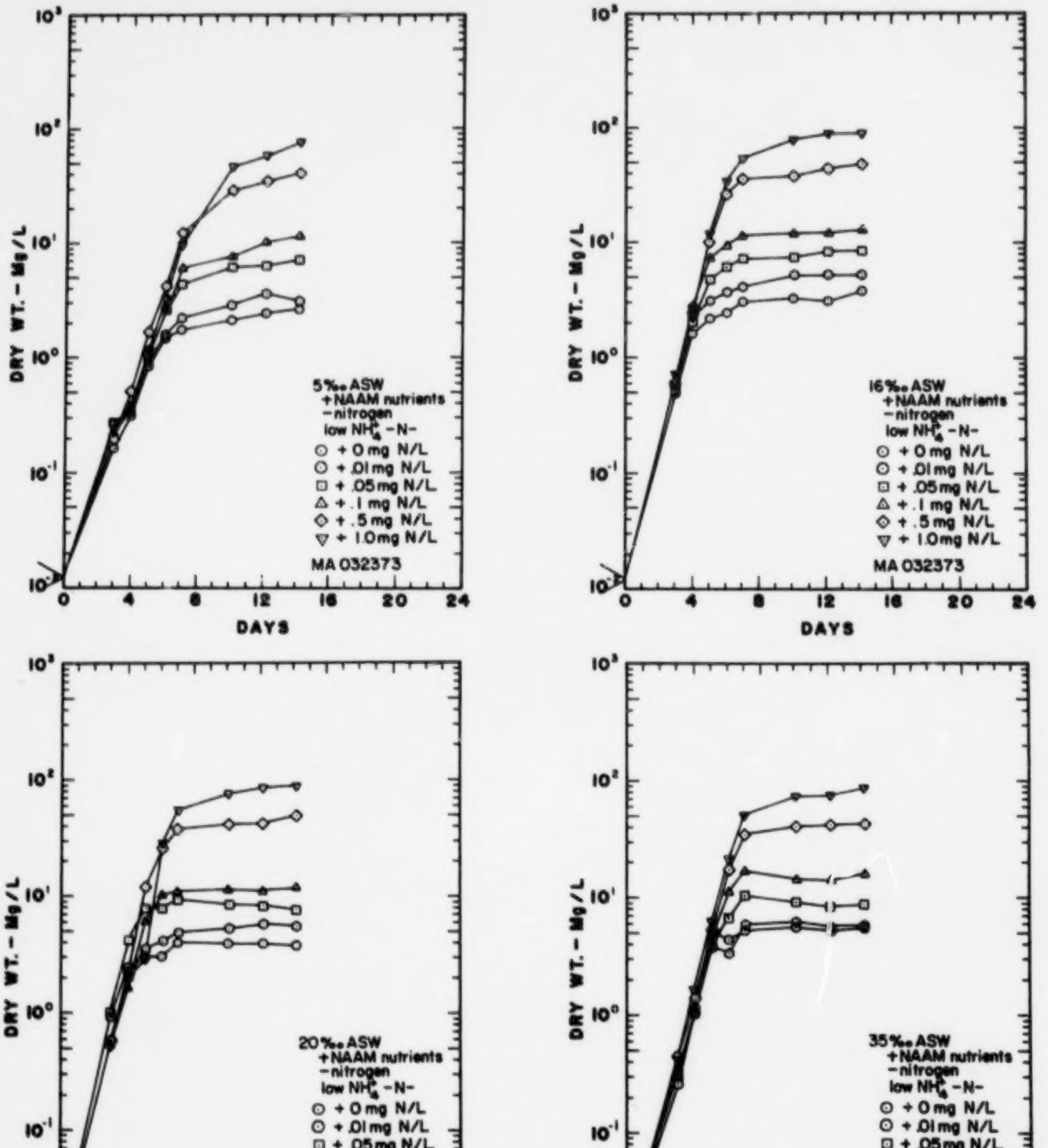


Figure 7. Growth response of Dunaliella tertiolecta

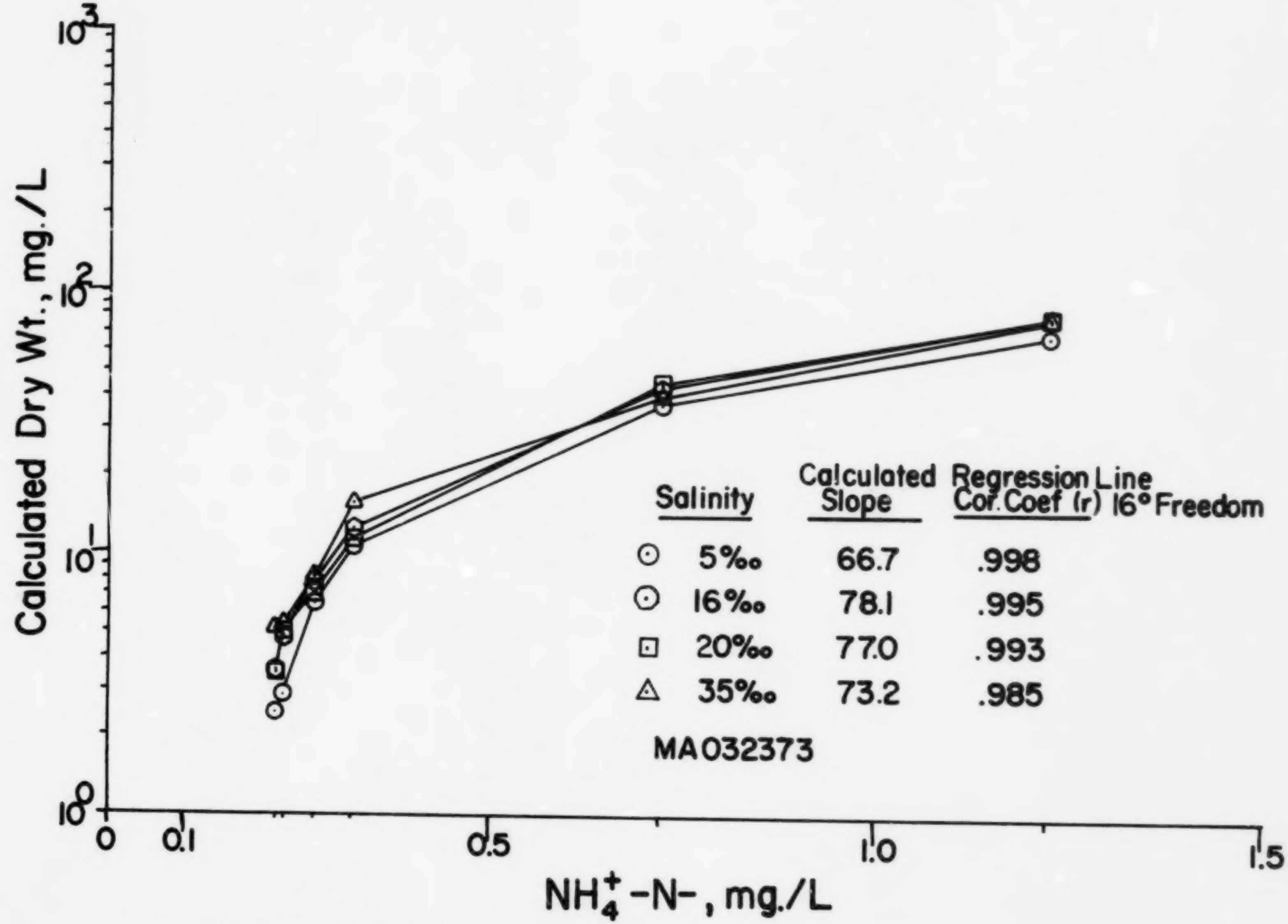


Figure 8. Growth response of *Dunaliella tertiolecta*, day 14

TABLE 2. BIOMASS PRODUCED PER UNIT OF NUTRIENT BY DUNALIELLA AT DAY 14 IN DEFINED MEDIA

	mg dry weight/µg of nutrient			P:N ratios	
Nutrient Salinity	P	NO_3^- - N	NH_4^+ - N	P:NO_3^- - N	P:NH_4^+ - N
5 ‰	0.557 ±0.158	0.0096 ±0.0014	0.0747 ±0.0075	1:58	1:7.5
16‰	0.930 ±0.240	0.0308 ±0.0394	0.0844 ±0.0058	1:30.2	1:11.0
20‰	1.170 ±0.164	0.0331 ±0.0379	0.0766 ±0.005	1:35.3	1:15.3
35‰	1.129 ±0.232	0.0315 ±0.0361	0.0765 ±0.0193	1:35.8	1:14.8
Average*	1.076	0.0318	0.0796	1:33.8	1:13.5

*Average is calculated from the 16 ‰, 20‰, and 35‰ data only.

Replication

One especially outstanding characteristic of the use of Dunaliella was the consistency of data replication. An example of this is the result of the investigation of NTA as a nitrogen source in natural water samples. NTA appeared to have little, if any, effect on the growth of Dunaliella, either stimulatory or inhibitory. In the experiment, the control and four levels of NTA (0.05 to 1.0 mg/l as N) with three replicates each gave a total of fifteen replicates for each of six natural waters (all Oregon coastal estuaries). In all cases, a t-test (13 degrees of freedom) showed no significant difference between dry weight from controls and samples spiked with varying levels of NTA. The normalized standard deviation for the entire run of 90 flasks was less than ± 15 percent.

Standard Inoculum Level

In order to approach natural phytoplankton population levels and avoid problems of nutrient carry-over in a healthy inoculum, the size of the inoculum was reduced from an initial 1,000 cells/ml (0.3 mg/l dry weight) to 100 cells/ml (0.03 mg/l dry weight). Although there was a detectable lag in growth of about two days, growth rate was unaffected and the final yield was virtually identical at day 10 or 12 (Figure 9). The inoculum was dispensed in a 1 ml volume.

Field Tests

The majority of field work has been done with water from the Yaquina River, an estuary at Newport, Oregon (Figure 10). It is relatively near and has been studied extensively by other EPA and Oregon State University personnel. Some samples were also obtained from several sites in Puget Sound and assayed in cooperation with the Washington State Department of Ecology.

The ability of Dunaliella to respond to low level nitrogen spikes in a natural water sample is shown in Figure 11, which illustrates the shift in potential nutrient limitation from nitrogen to phosphorus in a sample from the Toledo station in the Yaquina estuary. As the sample was presumed to be nitrogen limited, nitrate was added in incremental steps of 0.02, 0.1, 0.2, 0.5, and 1.0 mg/l as nitrogen to try and find the point at which all the phosphorus would be exhausted, shifting the sample to phosphorus limited growth (0.001 to 0.05 mg P/l and N + P combinations were also added in overall examination of the sample). The growth response at day 7 started to decline just below 0.5 mg/l total nitrogen, plateauing at about 23 mg dry wt/l with the 1.0 mg N/l spike.

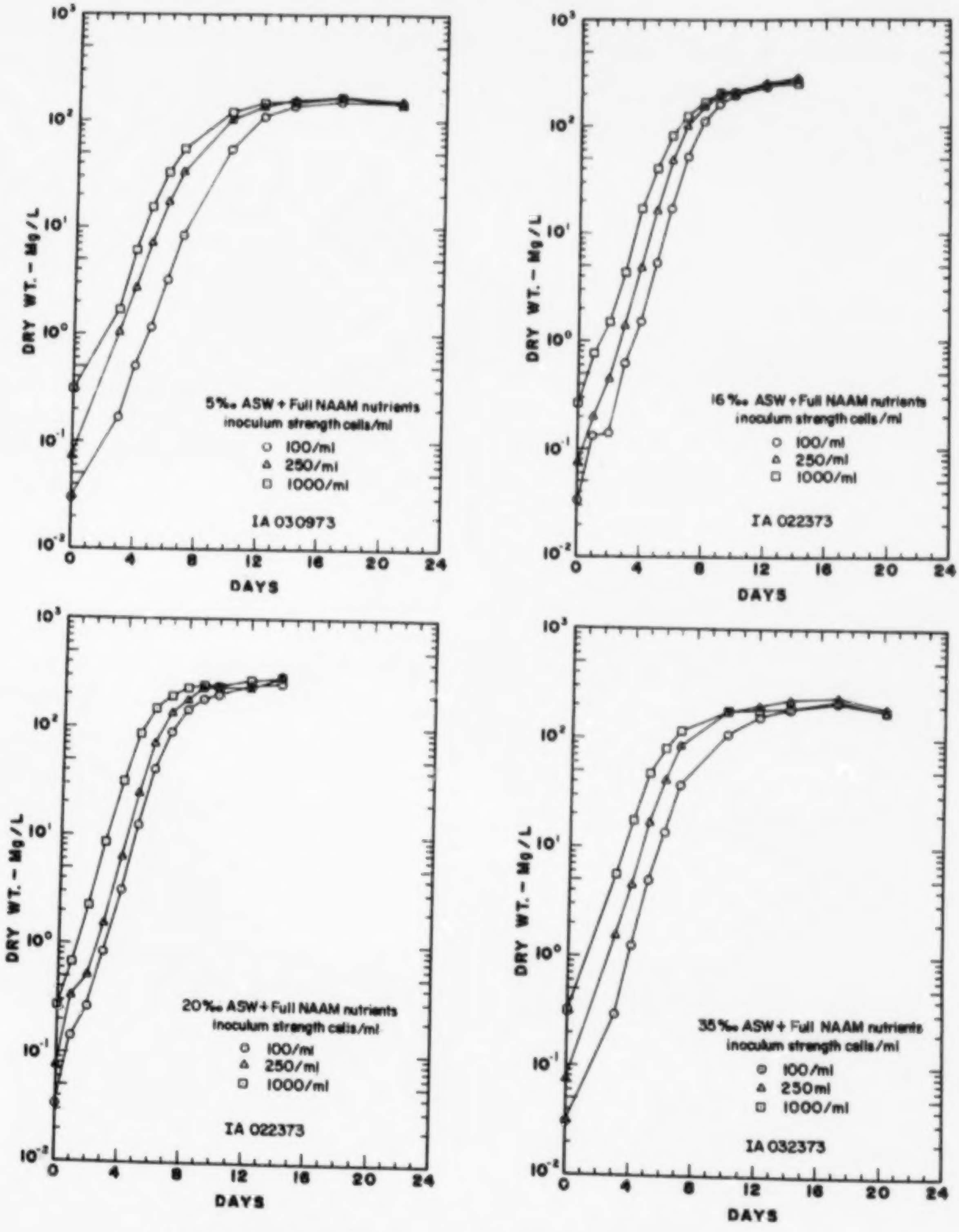


Figure 9. Growth response of Dunaliella tertiolecta.

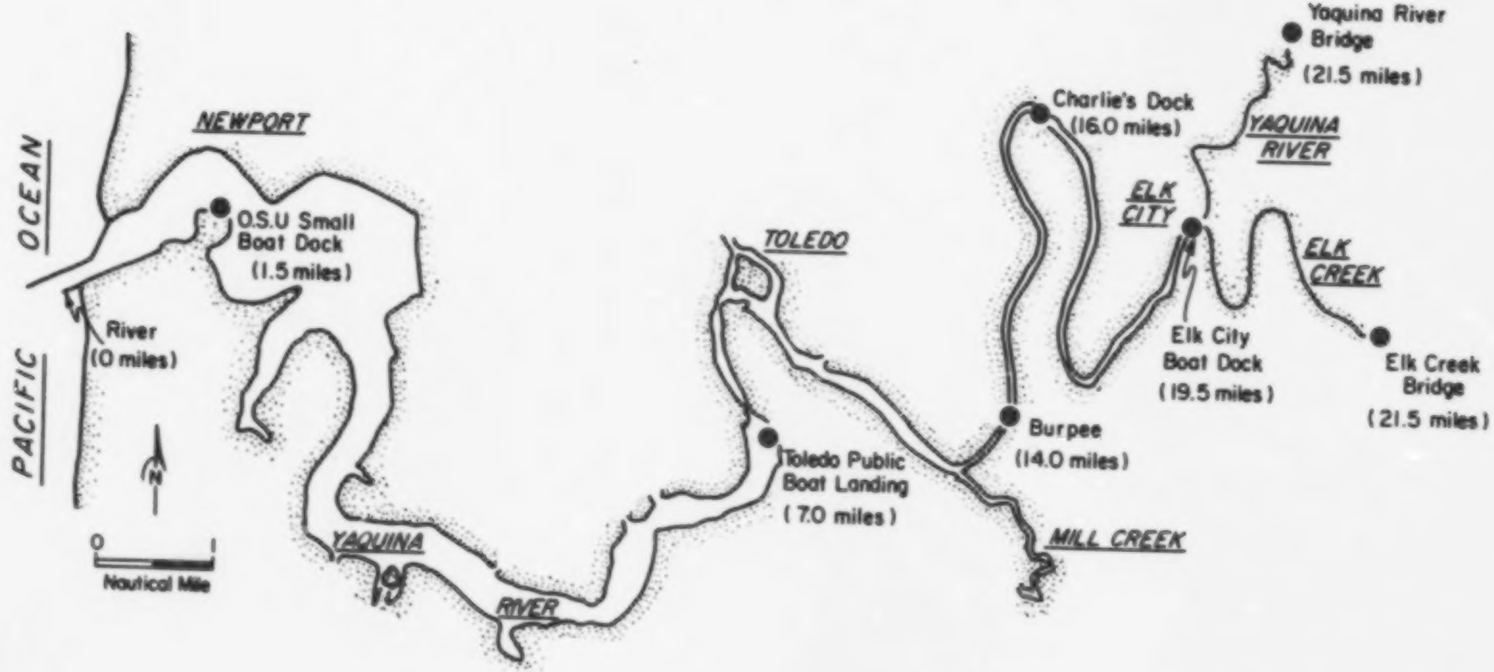

NEWPORT
OCEAN
PACIFIC
O.S.U Small Boat Dock (1.5 miles)
River (0 miles)
N
0
1
Nautical Mile
YAQUINA
RIVER
TOLEDO
Toledo Public Boat Landing (7.0 miles)
MILL CREEK
Burpee (14.0 miles)
Charlie's Dock (16.0 miles)
ELK CITY
Elk City Boat Dock (19.5 miles)
YAQUINA RIVER
Yaquina River Bridge (21.5 miles)
ELK CREEK
Elk Creek Bridge (21.5 miles)

Figure 10. Marine algal assay field sampling sites, Yaquina Estuary, Newport, Oregon.

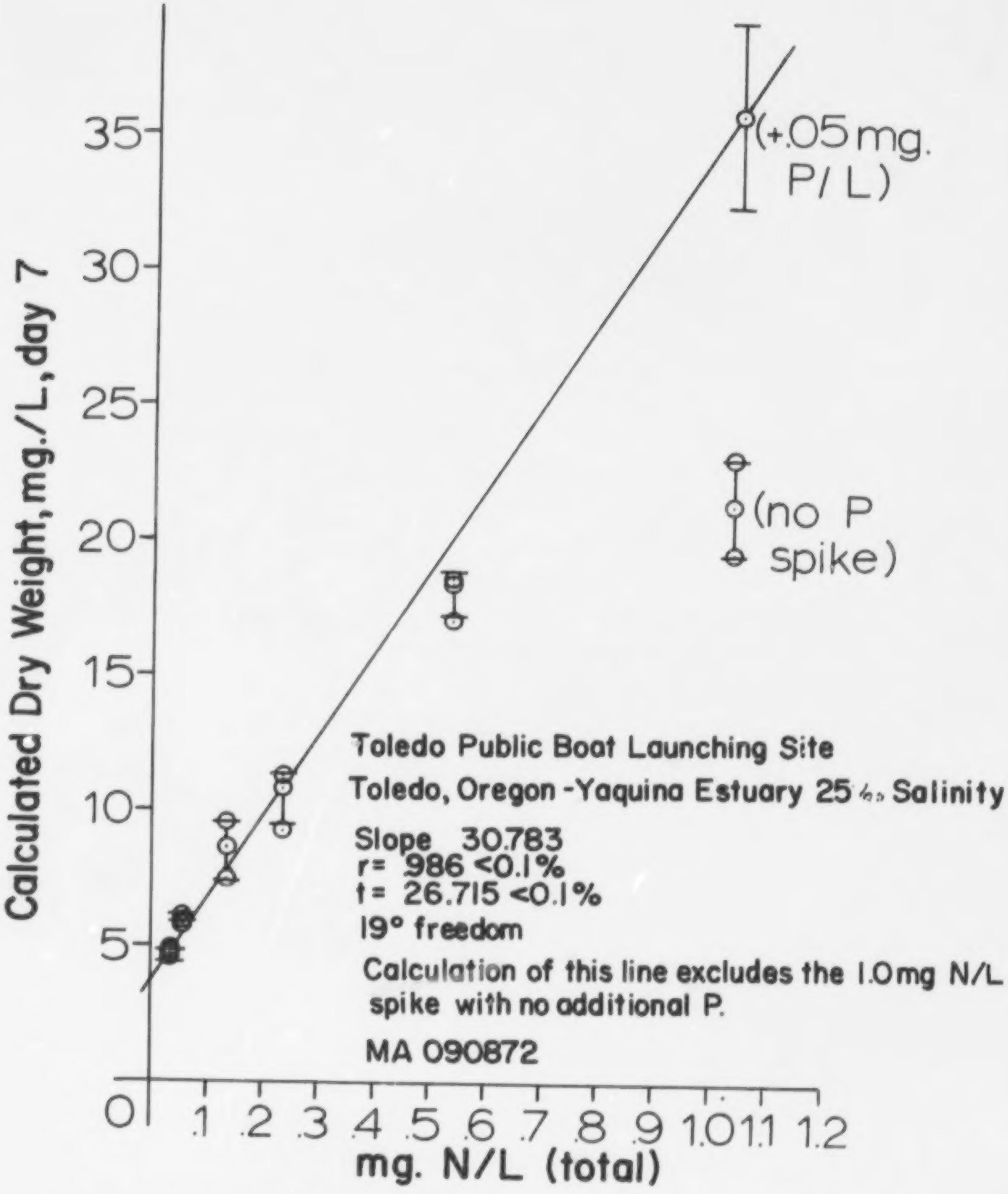


Figure 11. *Dunaliella* growth response to NaNO$_3$ spikes. Plot shows advent of phosphorus limitation, given enough nitrogen in nitrogen limited water.

The sample was at that point limited by phosphorus. To demonstrate this point, we examine the biomass produced by the 0.05 mg P + 1.0 mg N/l spike which yielded approximately 36 mg dry wt/l. If all points on the plot not limited by phosphorus (excludes the 1.0 μg N/l spike) are considered, a line of very good fit can be plotted ($r = 0.986$, $t = 26.7$, 19 degrees of freedom, significant at the 0.1% level) showing that about 97 percent (r^2) of the change in dry weight can be attributed to a change in the level of nitrogen. To show the advent of phosphorus limitation, a curve can be fitted (by the least squares power curve method) to the increasing N only spikes ($r = 0.987$), showing a plateau of approximately 23 mg dry wt/l defined by the 0.5 and 1.0 mg N/l spikes. The multiple levels of spikes show that when nitrogen is limiting, growth responses are linear with respect to the spike level until some other nutrient or physical factor becomes limiting.

Samples from Puget Sound were collected and assayed by personnel from the Washington State Department of Ecology and EPA Region X. Figure 12 compares the relative growth of the different samples. Day 10 dry weights plotted against inorganic nitrogen accounted for 83 percent of the variation in dry weight, indicating that nitrogen was probably the limiting nutrient factor for algal growth.

In a set of Yaquina Bay samples taken on November 1, 1972, one day after the onset of heavy winter rains, phosphorus was found to be the limiting nutrient at the Burpee sampling site. Downstream stations (Toledo and OSU Dock) appeared growth limited by nitrogen (Figures 13 and 14). The bioassay was repeated after a storage period of six weeks. Nitrogen limitation in the Toledo station was confirmed and the phosphorus limitation was reaffirmed, indicating that the apparent phosphorus limitation was real and was not a condition related to decreased salinity. It was instead probably caused by the increased nitrogen content of the rainwater runoff.

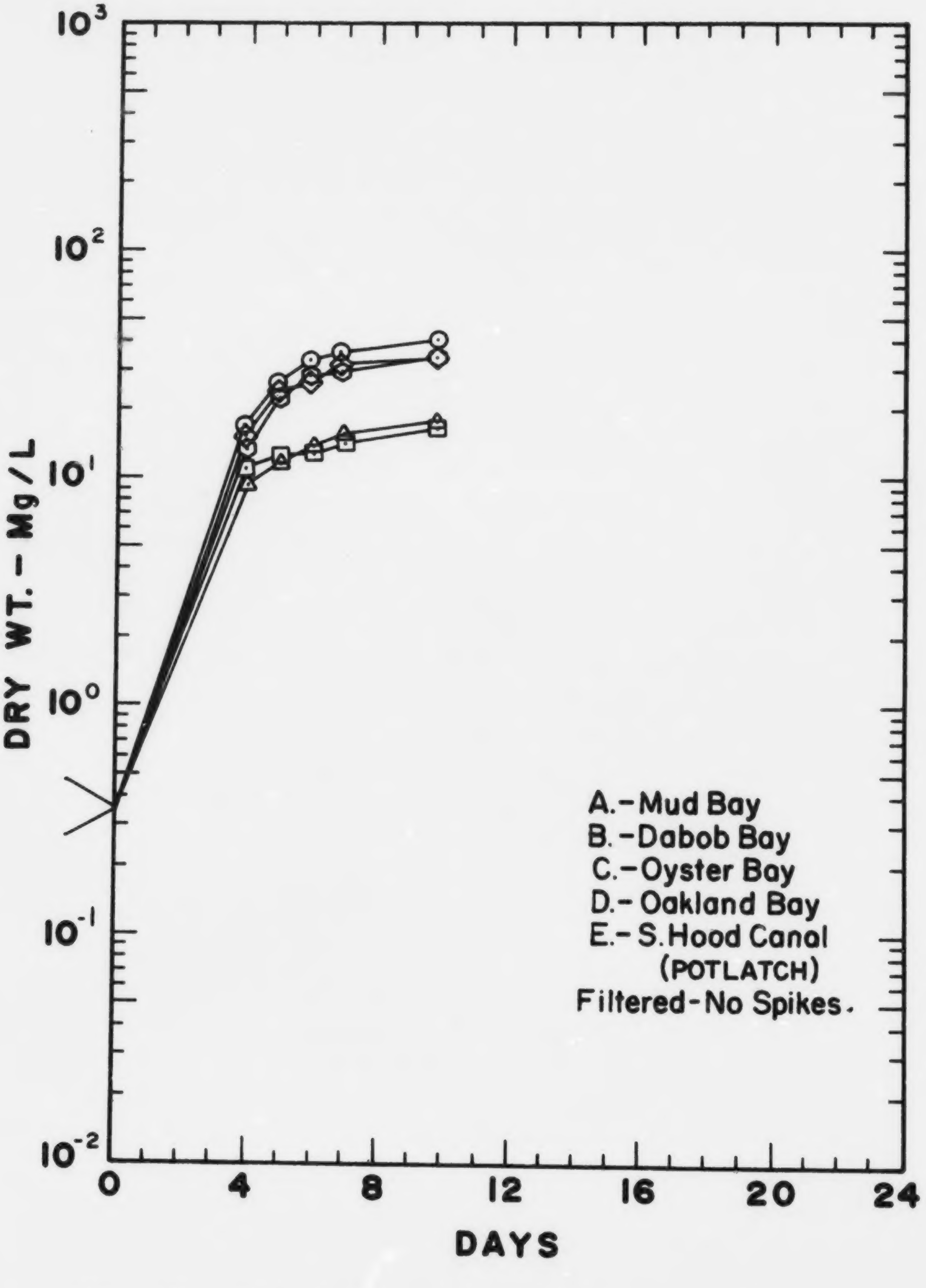
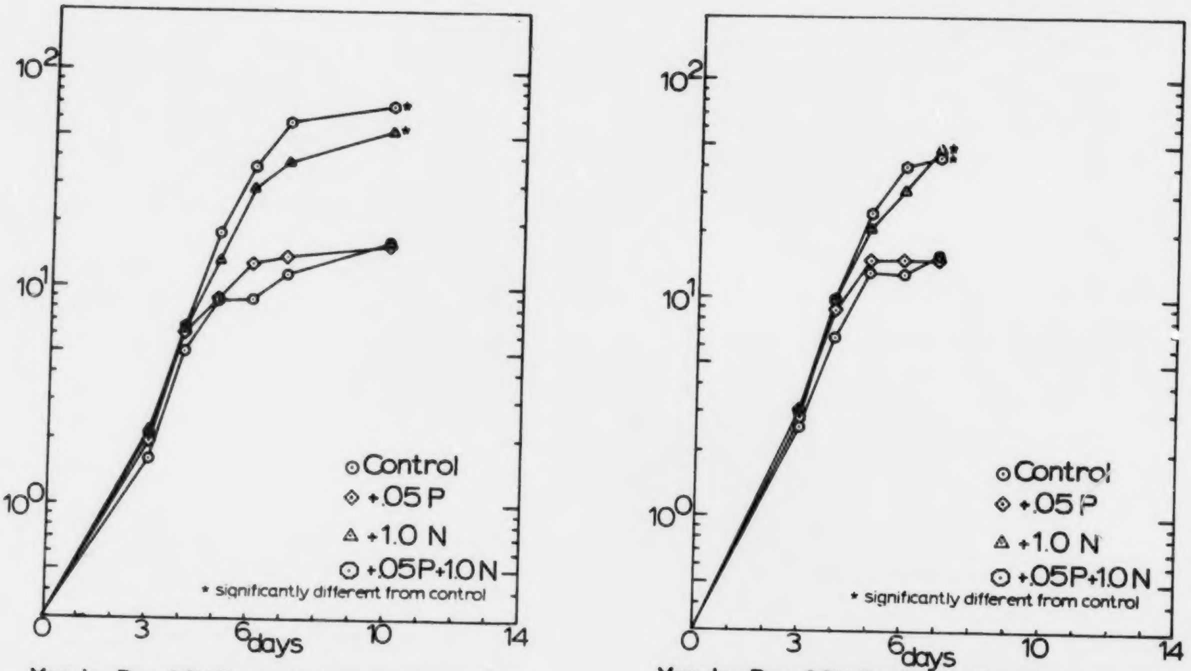
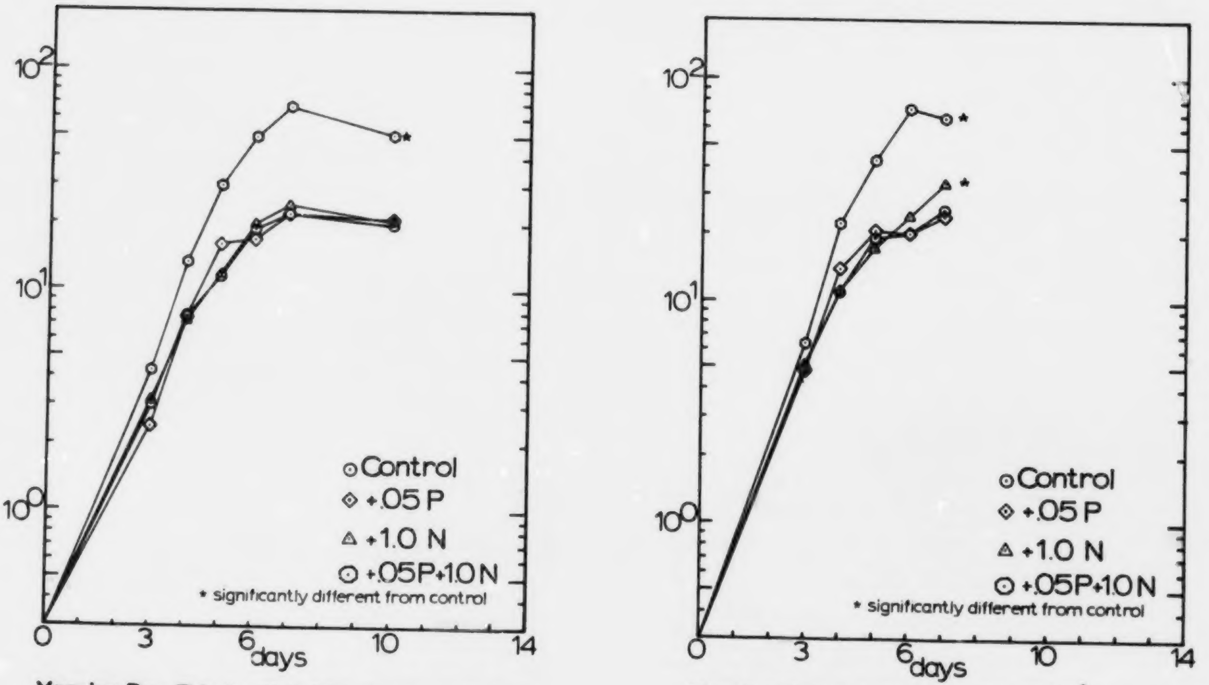


Figure 12. Algal assay of Puget Sound sites, Spring 1973.



Yaquina Bay, OSU Dock, 11/1/72 33.8‰ salinity

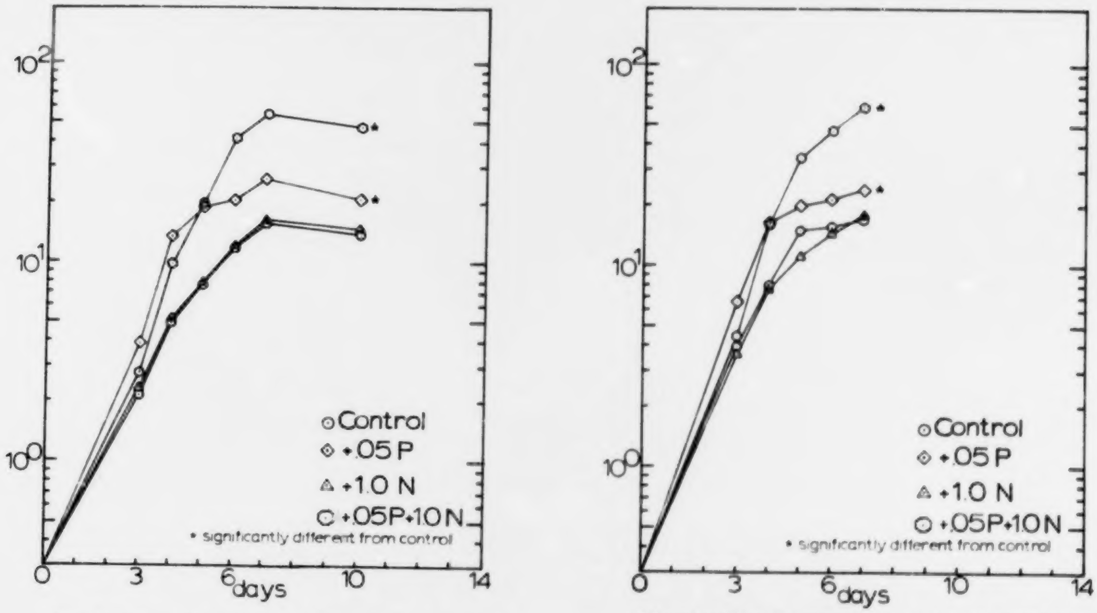
Yaquina Bay, OSU Dock, 11/1/72 33.8‰ salinity (sample stored 6 weeks)



Yaquina Bay, Toledo, 11/1/72 24‰ salinity

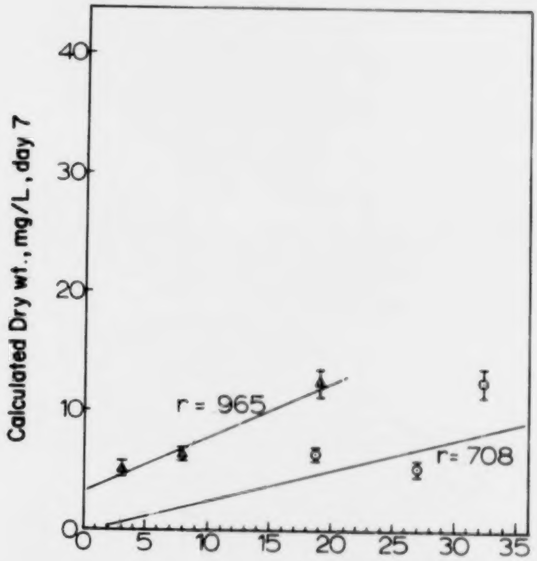
Yaquina Bay, Toledo, 11/1/72 24‰ salinity (sample stored 6 weeks

Figure 13. Growth response of Dunaliella tertiolecta.



Yaquina Bay, Burpee, 11/1/72, 17‰ salinity

Yaquina Bay, Burpee, 11/1/72 17‰ salinity (sample stored 6 weeks)



Yaquina Bay, 8 August 1972

△ = x 10^{-2} mg/L total N

○ = ‰ Salinity

Figure 14. Growth response of Dunaliella tertiolecta.

Figure 14 shows the plot of dry weights from controls of samples taken at the same stations in August, 1972. The data are plotted on two different axes, according to inorganic nitrogen content or according to salinity. A linear regression of the nitrogen line yields a correlation coefficient (r) of 0.965 (Student's t, 9.882, with 7 degrees of freedom, significant at the 0.1 percent level), indicating that approximately 93 percent (r^2) of the change in dry weight can be attributed to a change in the amount of nitrogen.

A similar statistical assessment of the salinity plot yields a correlation coefficient (r) of 0.708 (students t, 2.655, with 7 degrees of freedom, significant at the 5 percent level) indicating that only 50 percent (r^2) of the change in dry weight could be attributed to a change in salinity.

Samples were taken on June 28, 1973, in Yaquina Bay (Figure 10) from the OSU Dock station to the head of tidewater at Elk City, and approximately 2 miles farther up each of the two main tributaries, Elk River and Yaquina River. Salinities ranged from 30.8 °/₀₀ (49,300 µmho conductivity) to less than 2.8 °/₀₀ (down to 65 µmho) in the Yaquina River sample (see Table 3 for chemical analyses of samples [Environmental Protection Agency, 1971].

Given time to adjust physiologically, Dunaliella grew in these essentially freshwater situations and gave a statistically significant indication of the growth limiting nutrient (Figures 15 and 16). In this case, it is evident from the growth curves that nitrogen was the growth limiting nutrient at the OSU Dock Station (Figure 15). The situation at the Toledo station (14 °/₀₀ salinity) is somewhat indistinct, but a t-test shows that growth in the phosphorus spiked sample is significantly higher (at the 5 percent level) than the control and those spiked with nitrate or ammonia nitrogen. This indicates that phosphorus is the

Table 3. CHEMICAL PARAMETERS OF YAQUINA ESTUARY AND TRIBUTARY SURFACE GRAB SAMPLES, 28 June 1973, AT OR NEAR HIGH WATER, ON INCOMING TIDE

Parameter Sampling Site	OSU Small Boat Dock	Toledo Public Boat Landing	Burpee	Charlie's Dock	Elk City	Elk River, Upstream	Yaquina River, Upstream
NH_4^+ -N- mg/l	0.036	0.018	0.076	0.06	0.038	0.025	<0.001
NO_3^- -N- mg/l	0.120	0.129	0.416	0.430	0.498	0.315	0.560
Tot. sol. P mg/l	0.035	0.015	0.015	0.015	0.015	0.015	0.01
Ortho-P mg/l	0.03	0.013	0.011	0.019	0.009	0.011	0.008
Sulfate, mg/l	3100	896	256	10	3	<2.5	4
Sol. Fe, μg/l	240	100	100	140	140	160	140
Sol. Mn, μg/l	20	27	34	11	13	10	9
Salinity, °/oo	30.76	14.14	2.882	<2.84	<2.84	<2.84	<2.84
Conductivity, μmho	49,200	23,200	5,800	208	69	67	65
Tot. Sol. C, mg/l	24.5	15.8	9	5.4	4.7	4.8	4.5
Sol. reactive Si, mg/l	.68	2.2	4.6	4.8	5.25	5.4	5.5

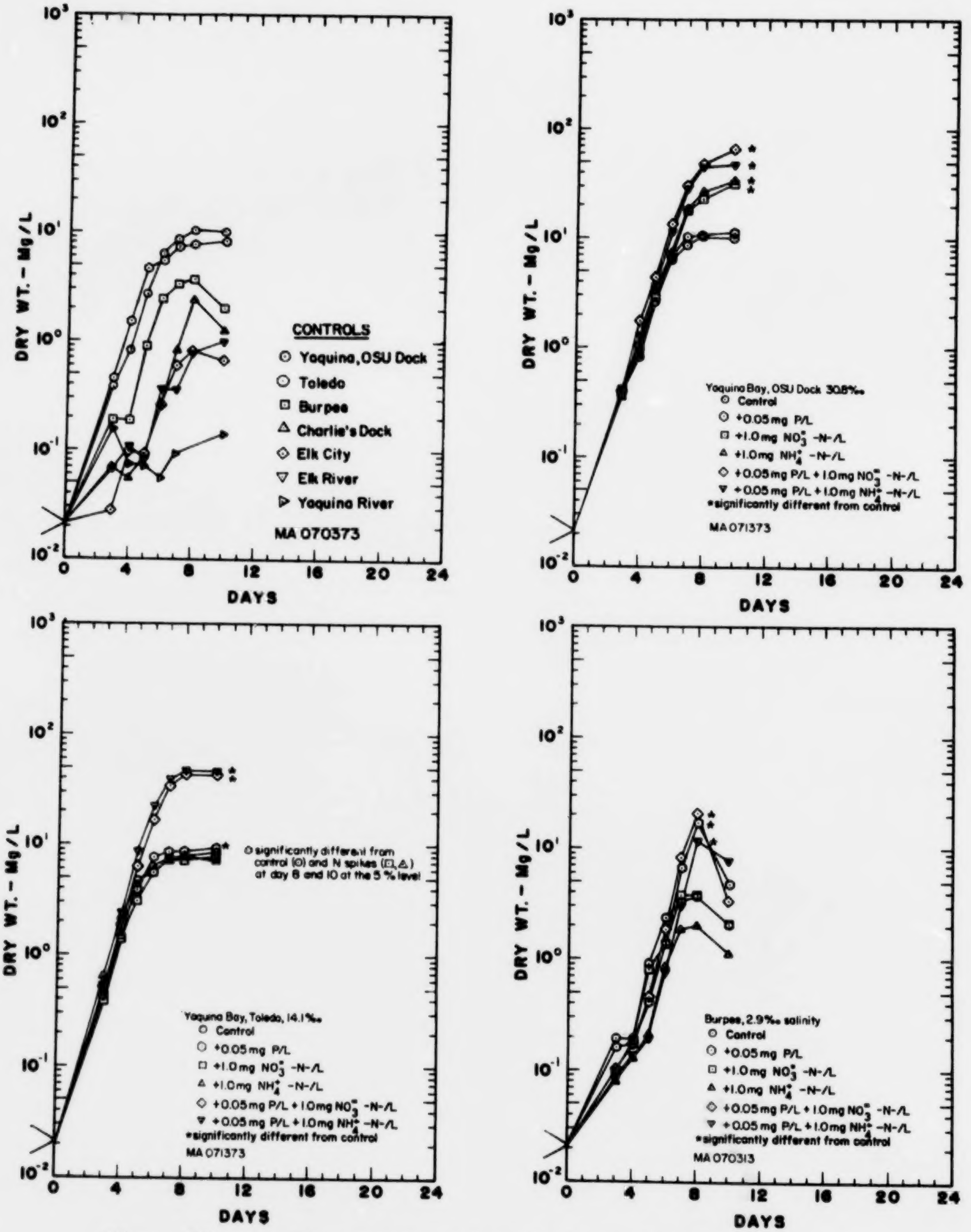


Figure 15. Growth response of *Dunaliella tertiolecta*.

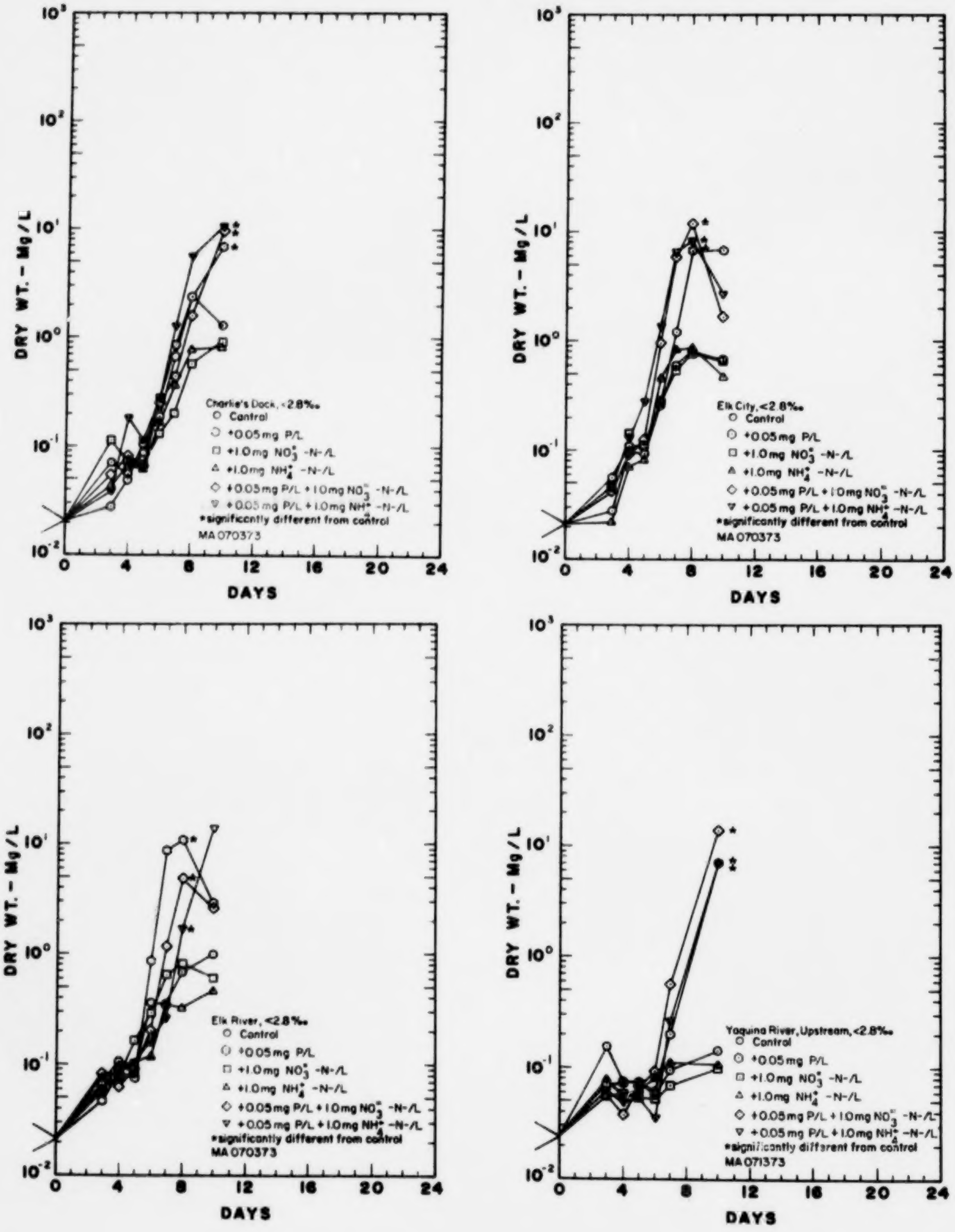


Figure 16. Growth response of Dunaliella tertiolecta.

growth limiting nutrient. At the Burpee station (Figure 15), phosphorus was shown to be the growth limiting nutrient (2.9 °/₀₀ salinity, 5800 μmho). The remainder of the stations, all essentially freshwater (208-65 μmhos conductivity), showed a marked lag in growth patterns. All samples responded in a statistically significant manner to phosphorus spikes, but not to nitrogen. Replication with Dunaliella was not as good in waters of very low salinities as in those of higher salinities, but more experimentation should yield a workable test protocol to establish acceptable replicability.

Five southern Oregon coastal estuaries (Figure 17) were sampled on July 25, 1973, from Coos Bay on the south to Yaquina Bay on the north. These estuaries, sampled on the flood tide at or near high water, were nitrogen limited with the exception of the Umpqua River. This sample, 11.6 °/₀₀ salinity, responded in a statistically significant manner to phosphorus spikes, but not to nitrogen spikes (Figures 18 and 19; see Table 4 for chemical analyses of samples [Environmental Protection Agency, 1971].

A non-marine application of the Marine Algal Assay Procedure using Dunaliella is exemplified by results obtained with samples of water from Abert Lake, a highly alkaline (50,000 μmho conductivity) drainage sink in southeastern Oregon. Attempts to assay this sample using Selenastrum capricornutum Printz of the Freshwater Algal Assay Procedure failed when the algal inoculum was killed by the high alkalinity. However, after an initial adjustment time lag, Dunaliella showed nitrogen to be the growth limiting nutrient (Figure 20).

SUMMARY

The effort to design and evaluate a marine version of the Algal Assay Procedure: Bottle Test has shown the suitability of Dunaliella tertiolecta Butcher (DUN clone) as a highly versatile and consistent bioassay organism for nutrient assessment in marine, estuarine, and some alkaline freshwater situations.

Table 4. CHEMICAL PARAMETERS OF 6 OREGON ESTUARY SAMPLES AFTER MEMBRANE FILTRATION

Surface Grab Samples, 25 July 1973,
on Incoming Tide at or near High Water

Sampling Site Parameter	Coos Bay at North Bend	Coos Bay at Horsefall Rd. Bridge	Umpqua River at Reedsport	Siuslaw River at Florence	Alsea River at Waldport	Yaquina River at Newport
NH_4^+ -N- mg/l	0.145	0.118	0.027	0.059	0.103	0.100
NO_3^- -N- mg/l	0.044	0.019	0.003	0.009	0.082	0.11
Total P mg/l	0.03	0.035	0.01	0.01	0.035	0.035
Ortho-P mg/l	0.028	0.028	<0.001	0.01	0.036	0.03
Sulfate mg/l	3200	3300	1500	2500	3400	3700
Sol. Fe, µg/l	240	240	80	120	240	240
Sol. Mn, µg/l	40	40	20	20	30	30
Salinity, °/∘∘	31.18	31.14	11.62	21.97	33.09	32.88
Conductivity, µmho	47,500	47,500	19,500	35,000	50,400	50,300
Tot. Sol. C, mg/l	25	26	13	18	25	25
Sol. reactive Si, mg/l	0.4	0.4	2.5	0.72	0.84	0.72

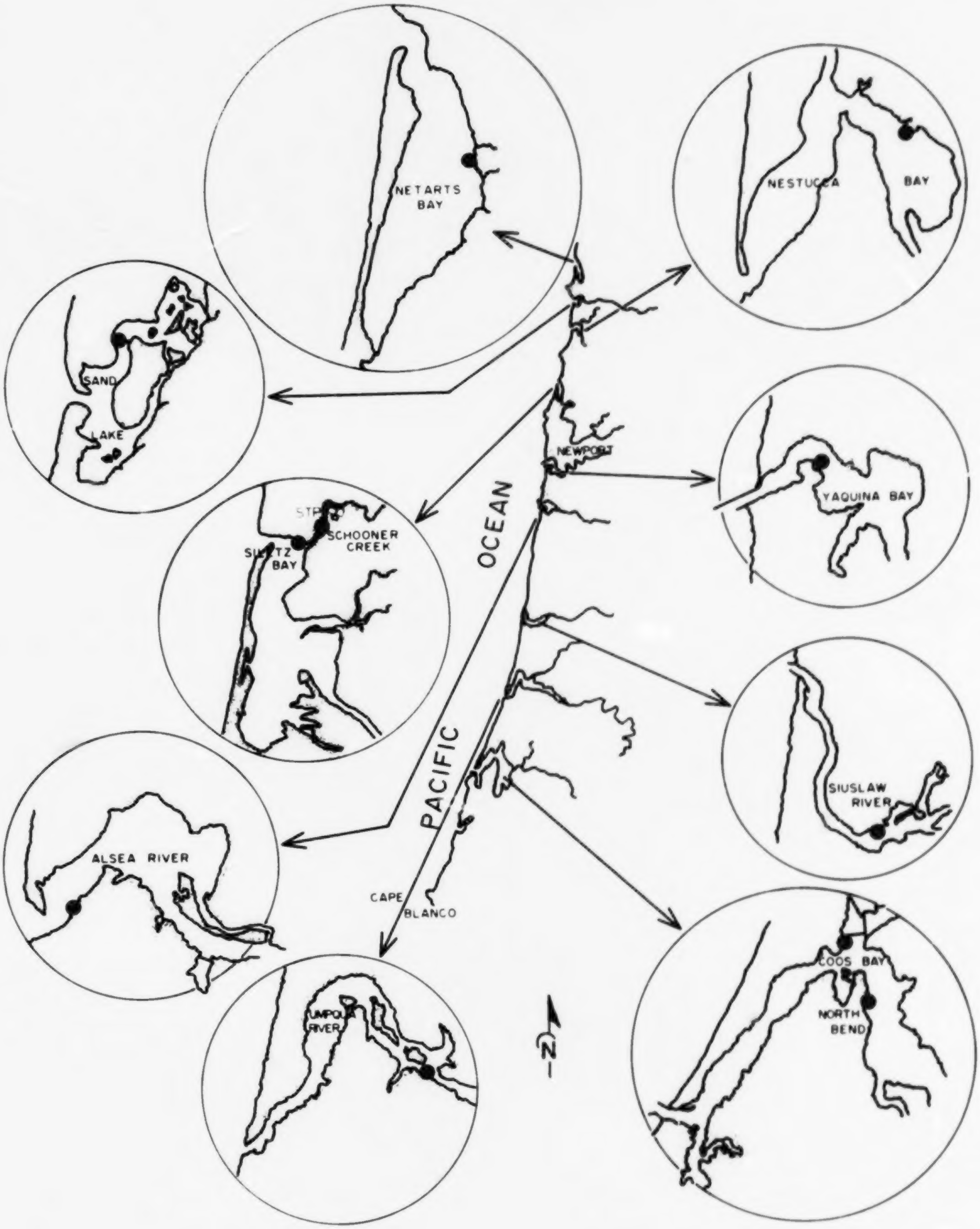

NETARTS BAY
NESTUCCA BAY
SAND LAKE
NEWPORT
YAQUINA BAY
STR
SCHOONER CREEK
SILETZ BAY
OCEAN
PACIFIC
SIUSLAW RIVER
ALSEA RIVER
CAPE BLANCO
COOS BAY
NORTH BEND
UMPQUA RIVER
N

Figure 17. Marine algal assay field sampling sites, Oregon coastal estuaries (not to scale).

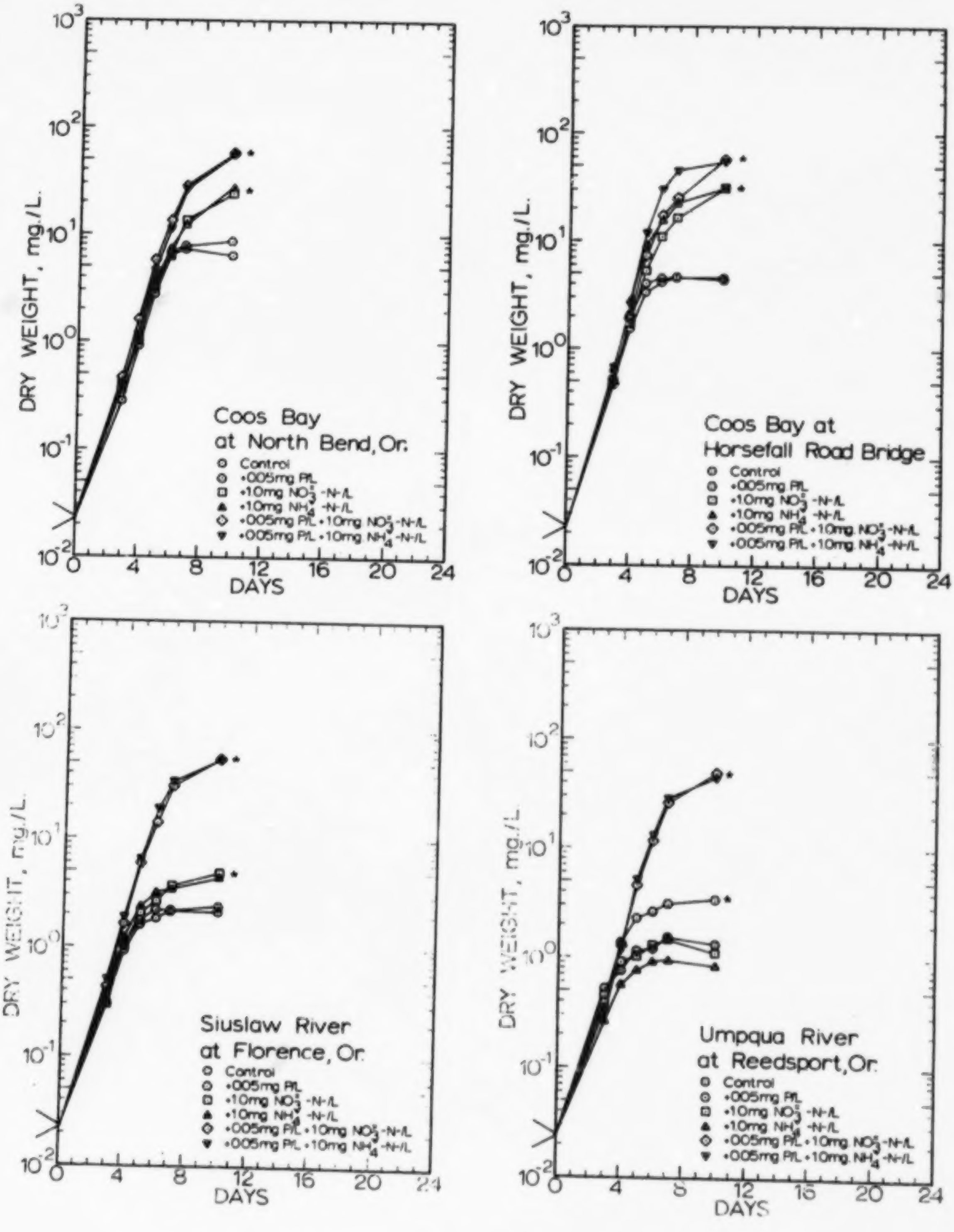


Figure 18. Growth response of Dunaliella tertiolecta.

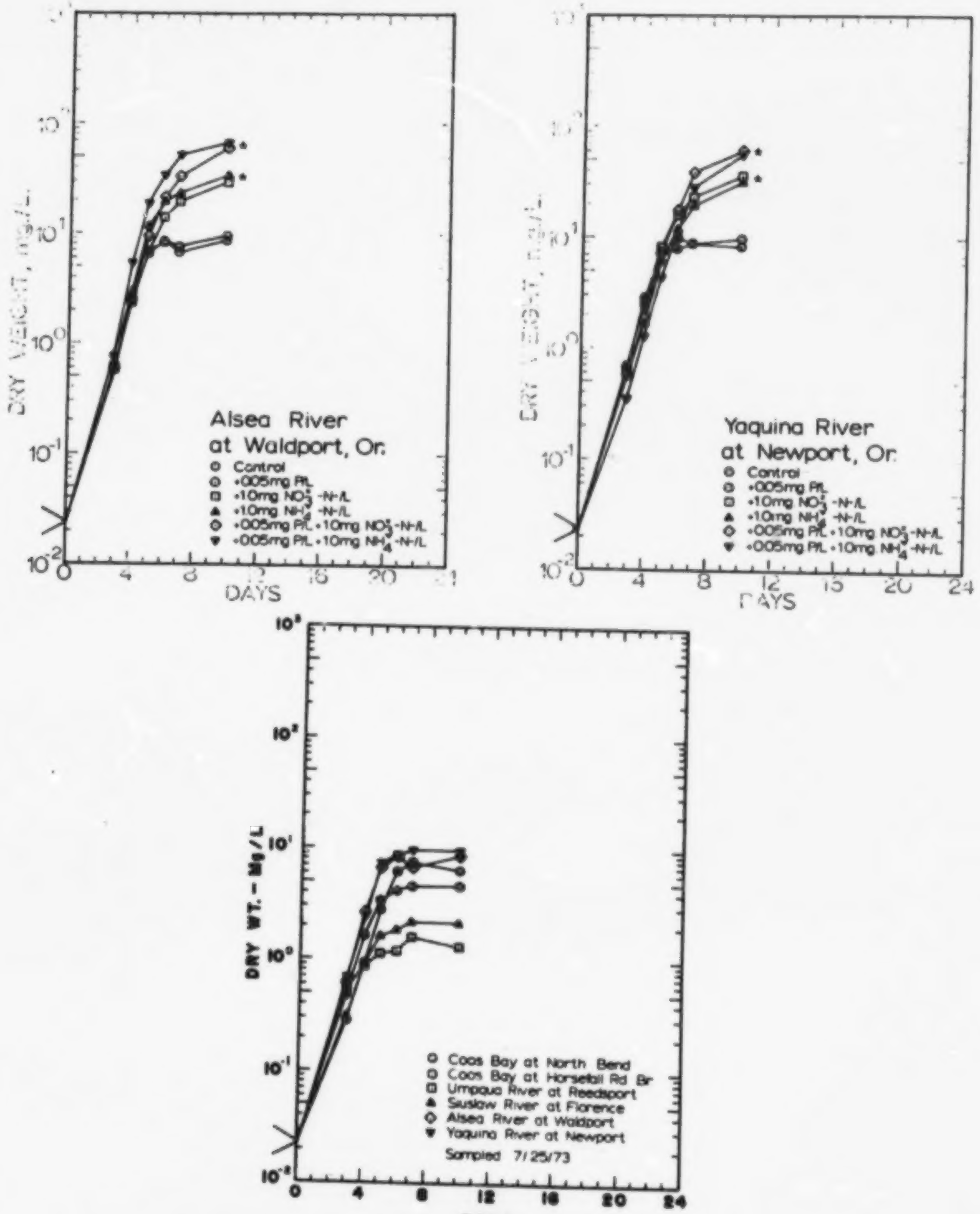


Figure 19. Growth response of *Dunaliella tertiolecta*.

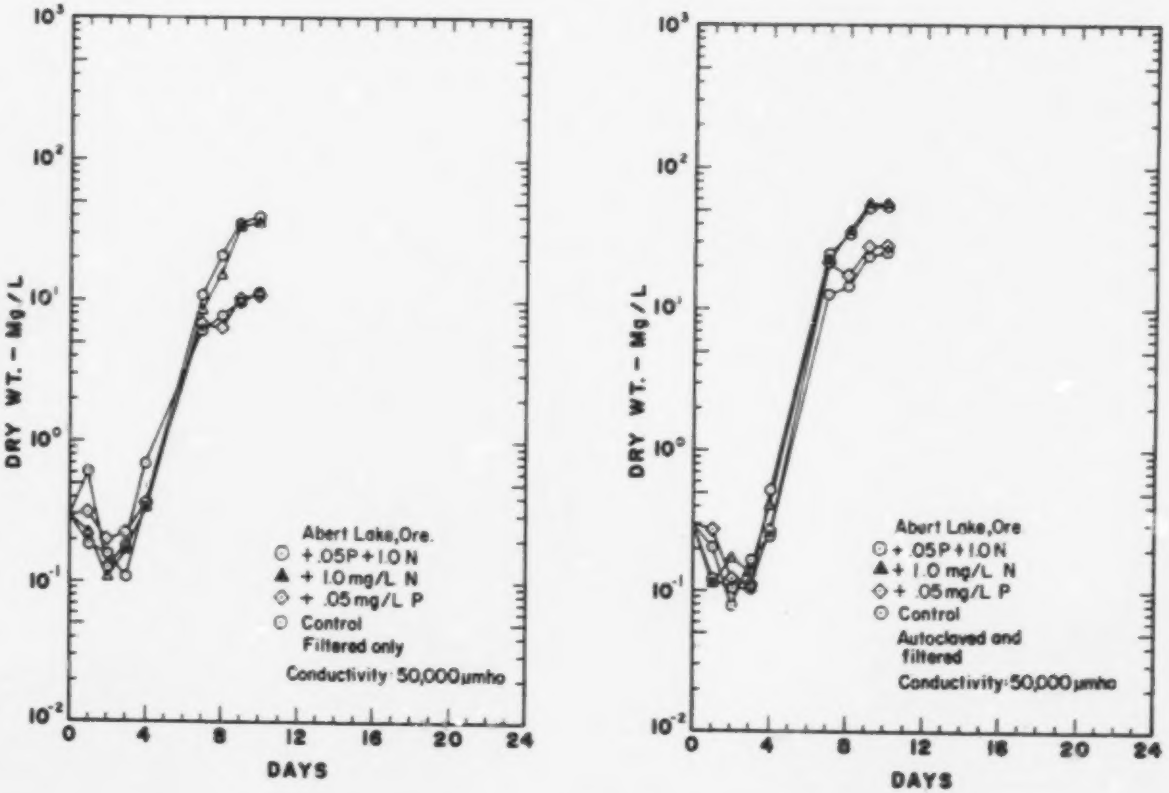


Figure 20. Growth response of Dunaliella tertiolecta in alkaline fresh water.

REFERENCES

Apollonia, S. 1973. Glaciers and Nutrients in Arctic Seas. Science 180:491-493.

Atkins, W. R. G. 1923. The Phosphate Content of Fresh and Salt Waters In Its Relationship to the Growth of Algal Plankton. J. Mar. Biol. Assoc. 13:119.

Burkholder, P. 1963. Some Nutritional Relationships Among Microbes of the Sea Sediments and Waters. In: Symposium on Marine Microbiology (C. H. Oppenheimer, ed.). Thomas, Springfield. pp. 133-150.

Dierberg, Forrest E. 1972. Development of an Algal Assay for Estuarine Water Quality. MS Thesis, University of North Carolina, Dept. of Environmental Sciences and Engineering.

Environmental Protection Agency. 1971. Methods for Chemical Analysis of Water and Wastes, 1971. Analytical Quality Control Laboratory, Water Quality Office, Cincinnati, Ohio. Publ. #16020---07/71.

Edmondson, W. T. and Y. H. Edmondson. 1947. Measurements of Production in Fertilized Salt Water. Jour. Mar. Res. 6:228-245.

Eppley, R. W., A. F. Carlucci, O. Holm-Hansen, D. Kiefer, J. J. McCarthy, and P. M. Williams. 1971. Evidence for Eutrophication in the Sea Near Southern California Coastal Sewage Outfalls, July, 1970. California Oceanic Fisheries Investigations Reports. UCSD 10P20-90.

Erickson, S. J., T. E. Maloney, and J. H. Gentile. 1970. Effect of Nitrilotriacetic Acid on the Growth and Metabolism of Estuarine Phytoplankton. J. Water Poll. Contr. Fed. 42(8) Pt. 2:R329-R335.

Fitzgerald, G. P. 1972. Bioassay Analysis of Nutrient Availability. In: Nutrients in Natural Waters (Herbert E. Allen and J. R. Kramer, eds.). Wiley Interscience, N.Y. pp. 147-170.

Glass, Gary E. 1973. Bioassay Techniques and Environmental Chemistry. Ann Arbor Science Publ., Inc. Ann Arbor, Mich.

Goldman, Joel C., Kenneth R. Tenore, and Helen I. Stanley. 1973. Inorganic Nitrogen Removal from Wastewater: Effect on Phytoplankton Growth in Coastal Marine Waters. Science 180:955-956.

Inman, Douglas L., and Birchard M. Brush. 1973. The Coastal Challenge. Science 181:20-31.

Kester, Dana R., Iver W. Duedall, Donald N. Connors, and Ricardo M. Pytkowicz. 1967. Preparation of Artificial Seawater. Limnol. and Oceanogr. 12(1):176-179.

Ketchem, Bostwick H. 1971. Population, Natural Resources, and Biological Effects of Pollution of Estuaries and Coastal Waters. In: Man's Impact on Terrestrial and Oceanic Ecosystems (William H. Mathews, Frederick E. Smith, and Edward D. Goldberg, eds.). MIT Press, Cambridge, Mass. pp. 59-70.

Larsen, D. P. 1973. Personal communication.

Lyman, J. and R. H. Fleming. 1940. Composition of Seawater. J. Mar. Res. 3:134-146.

McLachland, J. 1960. The Culture of Dunaliella tertiolecta Butcher - A Euryhaline Organism. Can. J. Microbiol. 6:367-379.

McPhee, Craig. 1961. Bioassay of Algal Production in Chemically Altered Waters. Limnol. and Oceanogr. 6:416-422.

Mitchell, Dee. 1973. Algal Bioassays for Estimating the Effect of Added Materials Upon the Planktonic Algae in Surface Waters. In: Bioassay Techniques and Environmental Chemistry (G. E. Glass, ed.). Ann Arbor Sci. Publ., Inc. Ann Arbor, Mich. pp. 153-158.

National Eutrophication Research Program. 1971. Algal Assay Procedure Bottle Test. Environmental Protection Agency. National Environmental Research Center, Corvallis, Oregon.

O'Sullivan, A. J. 1971. Ecological Effects of Sewage Discharge in the Marine Environment. In: A Discussion on Biological Effects of Pollution in the Sea (H. A. Cole, Organizer). Proc. Roy. Soc. Lond. B. 177(1048):331-351.

Paasche. E. 1971. Effect of Ammonia and Nitrate on Growth, Photosynthesis, and Ribuloseddiphosphate Carboxylase Content of Dunaliella tertiolecta. Physiol. Plant. 25:294-299.

Preston, A. and P. C. Wood. 1971. Monitoring the Marine Environment. In: A Discussion on Biological Effects of Pollution in the Sea (H. A. Cole, Organizer). Proc. Roy. Soc. Lond. B. 177(1048): 451-462.

Provasolie, L. 1963. Organic Regulation of Phytoplankton Fertility. In: The Sea, Vol. II (M. N. Hill, ed.). Wiley, N. Y. pp. 165-219.

Rudd, J. W. M., B. E. Townsend, and R. D. Hamilton. 1973. Discharge of Nitrilotriacetate (NTA) from Two Sewage Treatment Facilities Located in Mid-continental Climate. J. Fish. Res. Bd. Can. 30(7): 1026-1030.

Ryther, John H. 1954. The Ecology of Plankton Blooms in Moriches Bay and Great South Bay, Long Island, New York. Biol. Bull. 106(2):198-209.

Ryther, John H. and William M. Dunstan. 1971. Nitrogen, Phosphorus, and Eutrophication in the Coastal Marine Environment. Science 171:1008-1013.

Ryther, J. H. and R. R. L. Guillard. 1959. Enrichment Experiments as a Means of Studying Nutrients Limiting to Phytoplankton Production. Deep Sea Res. 6:65-69.

Skulberg, Olav. 1964. Algal Problems Related to the Eutrophication of European Water Supplies and a Bioassay Method to Assess Fertilizing Influences of Pollution on Inland Waters. In: Algae and Man (D. F. Jackson, ed.). Plenum Press, N. Y. pp. 262-299.

Sturm, R. N. and A. G. Payne. 1973. Environmental Testing of Trisodium Nitrilotriacetate: Bioassays for Aquatic Safety and Algal Stimulation. In: Bioassay Techniques and Environmental Chemistry (G. E. Glass, ed.). Ann Arbor Science Publ., Inc. Ann Arbor, Mich.

Smayda, T. J. 1970. Growth Potential Bioassay of Water Masses Using Diatom Cultures: Phosphorescent Bay (Puerto Rico) and Caribbean Waters. Helgol. Wiss. Meeresunters. 20:172-194.

Tallqvist, Torsten. 1973a. Algal Assay Procedure (Bottle Test) at the Norwegian Institute for Water Research. In: Algal Assays in Water Pollution Research: Proceedings from a Nordic Symposium, Oslo, 25-26 October, 1972. Nordforsk. Secretariat of Environmental Sciences, Helsinki. Publ. #1973-2. pp. 5-17.

Tallqvist, Torsten. 1973b. Use of Algal Assay for Investigating a Brackish Water Area. In: Algal Assays in Water Pollution Research: Proceedings from a Nordic Symposium, Oslo, 25-26 October 1972. Nordforsk. Secretariat of Environmental Sciences, Helsinki. Publ. #1973-2. pp. 111-123.

Tarzwell, C. M. 1971. Bioassays to Determine Allowable Waste Concentrations in the Aquatic Environment. In: A Discussion on Biological Effects of Pollution in the Sea (H. A. Cole, Organizer). Proc. Roy. Soc. Lond. B. 177(1048):279-285.

Taylor, Barrie F., Chun C. Lee, and John S. Bunt. 1973. Nitrogen-fixation Associated with the Marine Blue-green Alga, Trichodesmium, as Measured by the Acetylene-reduction Technique. Archiv. Mikrobiol. 88:205-212.

Thomas, William H. 1970. Effect of Ammonium and Nitrate Concentration on Chlorophyll Increases in Natural Tropical Pacific Phytoplankton Populations. Limnol. and Oceanogr. 15(3):386-394.

Thomas, William H. 1971. Effects of Nutrients on Growth of Algae; Tropical Oceanic Phytoplankton. Final Report to National Science Foundation. IMN Reference 71-9: Scripps Inst. Oceanogr.

Tyler, John E. 1973. Applied Radiometry. Oceanogr. Mar. Biol. Ann. Rev. 11:11-15.

Waite, Thomas and Ralph Mitchell. 1972. The Effect of Nutrient Fertilization on the Benthic Algae Ulva lactuca. Zeit. Botanica Marine 15(3):151-156.

Welch, Eugene B. 1968. Phytoplankton and Related Water-quality Conditions in an Enriched Estuary. J. Water Poll. Contr. Fed. 40(10):1711-1727.

GROWTH REQUIREMENTS OF *ENTEROMORPHA COMPRESSA* AND *CODIUM FRAGILE*

Richard L. Steele

INTRODUCTION

Enteromorpha compressa, other species of *Enteromorpha*, and members of the Ulvales are known to grow abundantly in areas where organic pollution is rampant (Perkins and Abbot, 1972; Levring, et al., 1969) and where salinity changes occur frequently. Research on *E. compressa*, a world-wide species, was begun with the purpose of developing a rapidly growing alga that could be used to detect effects of pollution and also become an easily manipulated bioassay tool. *Codium fragile*, a recent invader to the East Coast, has also become an obnoxious pest. First described from Long Island, New York, in 1957, it has become one of the most common sea weeds that one encounters along eastern shores from Maine to New Jersey. In Shinnecock Bay, Long Island, standing crops of 11,000 grams/square meter have been reported (Moeller, 1969). Aside from ecological imbalances encountered, the most deleterious effects are on the shellfish industry. Scallops, mussels, and oysters are no match for the buoyant plants that are swept by tides and currents and are subsequently deposited on shore where they die (Ramus, 1971). Control of both of these plants is dependent on knowledge of the biology of the plant.

MATERIALS AND METHODS

For this study, clean, actively growing plants of *Codium fragile* were obtained from either Newport, R.I., or Stonington Harbor in Connecticut. Plants of *Enteromorpha compressa* were obtained at low tide from

Charlestown Pond, Charlestown, R.I. Cultures of unialgal *E. compressa* and *C. fragile* were started by growing in unenriched sea water, with GeO_2 (6-10 mg/L) and 10 ml penicillin G per liter (100,000 units per ml), added to retard growth of diatoms (Lewin, 1966), bluegreen algae and bacteria. All cultures of both species were started with tips 1 cm long. Plants were grown at a day length of 16 hr./8 hr. light/dark cycle at 18°C ± 2°C. Illumination was 450-600 ft. C under cool white fluorescent lamps and the culture medium was changed every 4 days.

Experimental media is a formulation, TM-6, devised at the National Marine Water Quality Laboratory for trace metal studies and is of the following formulation, Table 1. In all cases silicon was omitted from the medium. For appropriate experiments, one or several of the ingredients were omitted. In experiments dealing with trace metals, the medium was further treated by passing through columns containing the sodium form of carefully purified (Davey, *et al*., 1970) chelex-100 (Bio-Rad). Metals were then added back in known concentrations for each experiment.

Other enrichments were tested by adding back to media deficient in a particular ion. Phosphorus was removed from the artificial sea water by treating with an excess of $FeCl_3$ (100 mg/L) and subsequently filtering the media through .22 µ Millipore filters.

Light, temperature, and salinity preference studies were performed in sea water that had been passed through the chelex-100 column. Temperature studies were done over the range of 0° to 30°C. and light experiments were done over the range of 50 ft. C to 1200 ft. C. For both species, growth was measured as increase in length of the excised tips 1.0 cm original length. All transfers were made with teflon coated forceps, rinsed after each transfer with 18 mohm deionized water.

Table 1. FORMULATION OF GROWTH MEDIUM TM-6

	Compound	Stock Solution	Per Liter
1.	Na Cl	21 g/100 ml	100 ml
2.	K Cl	.7 g/10 ml	10 ml
3.	$Mg\ SO_4\cdot 7H\ O$	12 g/100 ml	100 ml
*4.	Ca (as Cl)	1 g/100 ml	25 ml
5.	$Na\ NO_3$	71 mg/ml	1 ml
6.	K_2HPO_4	12.8 mg/ml	1 ml
*7.	$Na_2SiO_3\cdot 9H_2O$	3.075 mg/ml	10 ml
8.	Fe (as Cl)	1 mg/ml	.1 ml
9.	Vitamins		
	Thiamine HCl	.1 mg/ml	
	Biotin each ml contains	.5 μg/ml	
	B_{12}	.5 μg/ml	
10.	Trace Metals (TM-Metals)**		
	1 ml of stock solution contains the following:		
	$Cu\ SO_4\cdot 5H_2O$ .0098 mg	(0.0025 mg Cu)	
	$Zn\ SO_4\cdot 7H_2O$ .022 mg	(0.005 mg Zn)	
	$Co\ Cl_2\cdot 6H_2O$ .010 mg	(0.0025 mg Co)	
	$Mn\ Cl_2\cdot 4H_2O$ .180 mg	(0.05 mg Mn)	
	$Na_2Mo\ O_4\cdot 2H_2O$ .0063 mg	(0.0025 mg Mo)	
	H_3BO_3 1.140 mg	(0.2 mg B)	
11.	Adjust Ph		7.8
12.	TRIS (pH 7.8, 5% solution)		10 ml
13.	Autoclave		

*pH - 7.8

**Guillard, _et_ _al_., 1962.

RESULTS

Temperature and Illumination

Plants of *Codium fragile* grew most rapidly at 300 to 600 ft. C at 15-22°C. At other temperatures plants grew slower but at these temperatures, growth was still most rapid at 350-600 ft. C (Figure 1). When illumination was held constant at 600 ft. C and temperature was varied, best growth occurred at 16-20°C. (Figure 2). Above and below this range, growth was reduced. Plants of *Enteromorpha compressa* grew most rapidly at 300-500 ft. C when the temperature was held constant at every temperature regime tested (Figure 3). However, when the light intensity was held constant at 300 ft. C most rapid growth occurred at 20-25°C (Figure 4).

Salinity

Growth of *C. fragile* and *E. compressa* were much different at the range of salinities tested, i.e. 0-60 ‰. *E. compressa*, on the other hand, showed new growth nearly equal at 5 ‰ to 25 ‰ salinity, with a gradual decrease at higher salinities (Figure 5).

The nutrients phosphorus and nitrogen were varied over a wide range for both *C. fragile* and *E. compressa*. Nutrient requirements differ quite markedly for the two species. Growth of *Codium* was not appreciably increased by increasing the amounts of phosphorus and nitrate. One-tenth the amount of phosphorus and nitrogen used in TM-6 produced growth equal to the amount normally added (Figures 6 & 7). Conversely, *E. compressa* was definitely stimulated by higher amounts of both phosphorus and nitrogen (Figures 8 & 9).

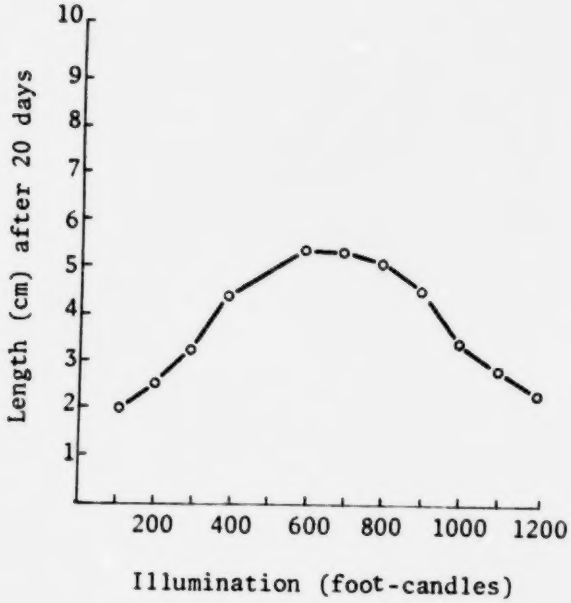


Figure 1. Increase in length of Codium fragile tips at various intensities of illumination (in ft. c). Temperature 18° C.

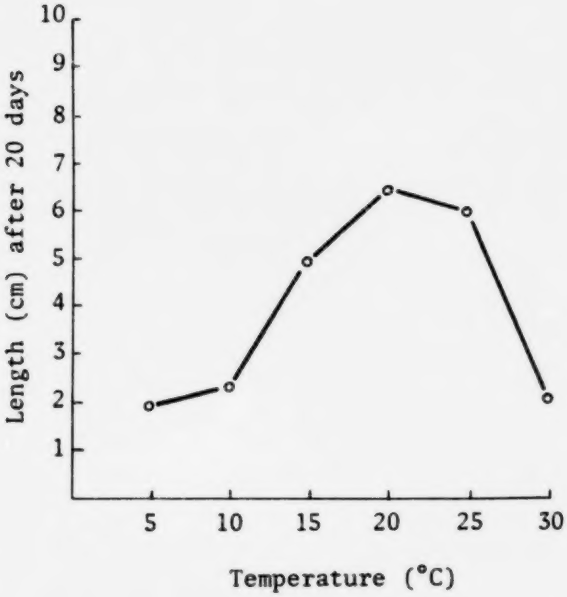


Figure 2. Increase in length of Codium fragile tips at various temperature regimes from 5°C. to 30°C. Illumination 600 ft. c.

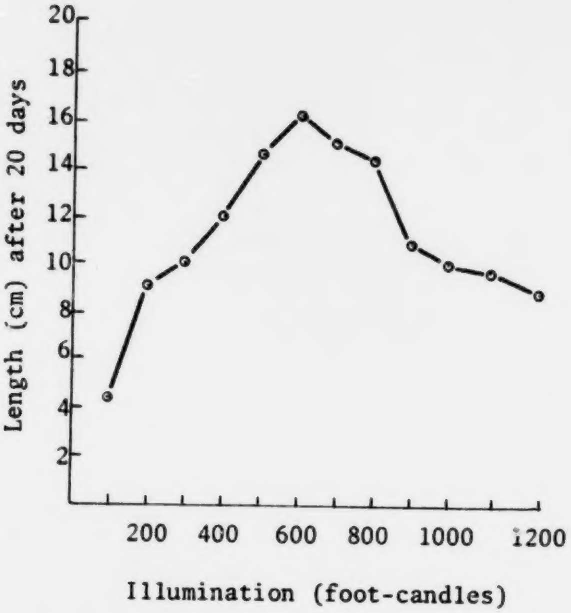


Figure 3. Increase in length of Enteromorpha compressa tips at various intensities of illumination (in ft. c). Temperature 20°C.

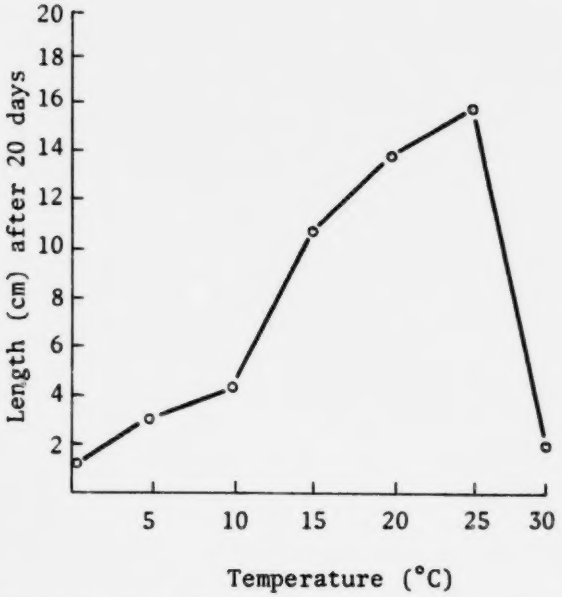


Figure 4. Increase in length of Enteromorpha compressa tips at various temperature regimes from 5°C. to 30°C. Illumination 300 ft. c.

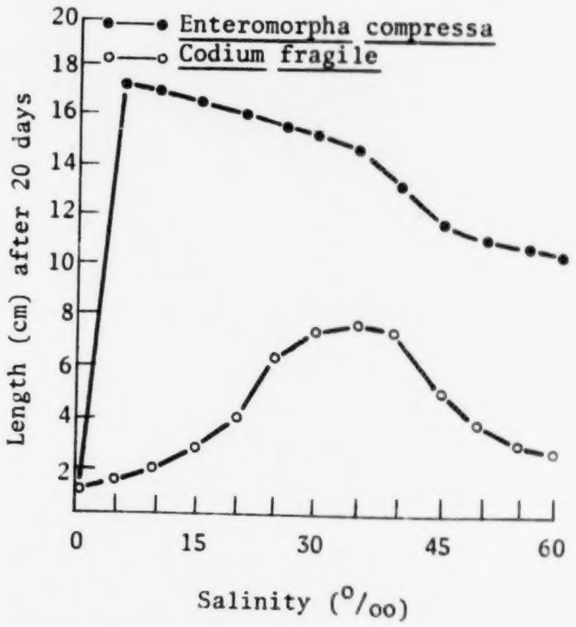


Figure 5. Increase in length of tips of Enteromorpha compressa (•—•) and Codium fragile (o—o) placed in sea water of various salinities. Temperature 20°C. Illumination 400 ft. C.

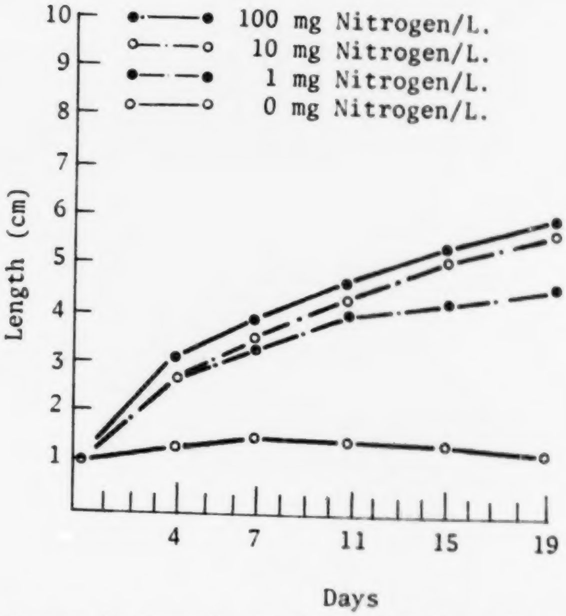


Figure 6. Increase in length of tips of Codium fragile placed in media containing increasing amounts of nitrogen. Temperature 20°C. Illumination 400 ft. C.

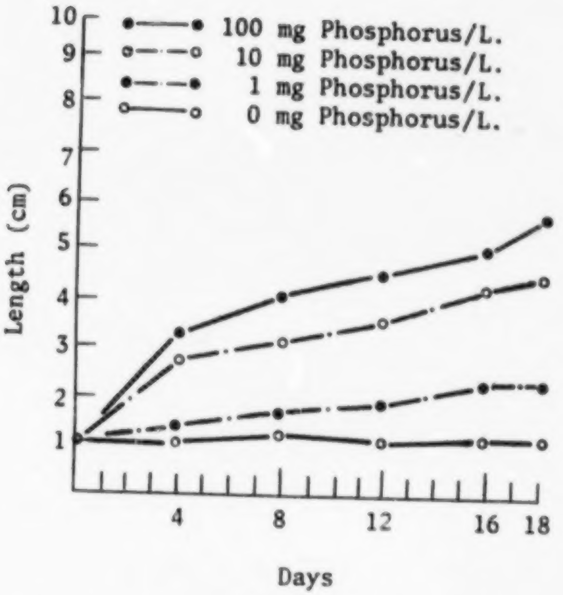


Figure 7. Increase in length of tips of Codium fragile placed in media containing increasing amounts of phosphorus. Temperature 20°C. Illumination 400 ft. C.

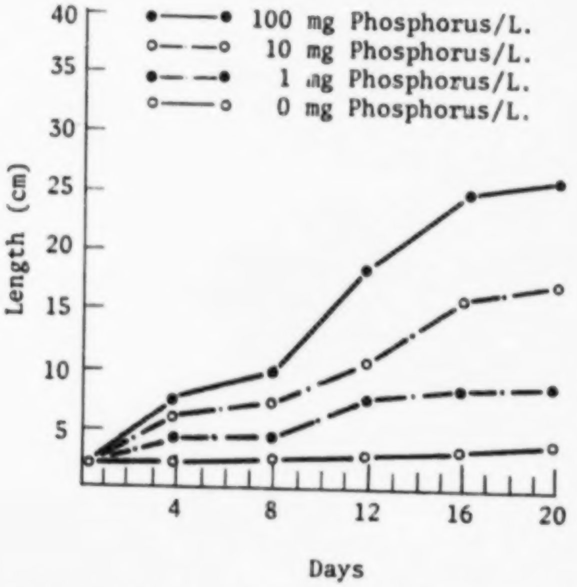


Figure 8. Increase in length of tips of Enteromorpha compressa placed in media containing increasing amounts of phosphorus. Temperature 20°C. Illumination 400 ft. C.

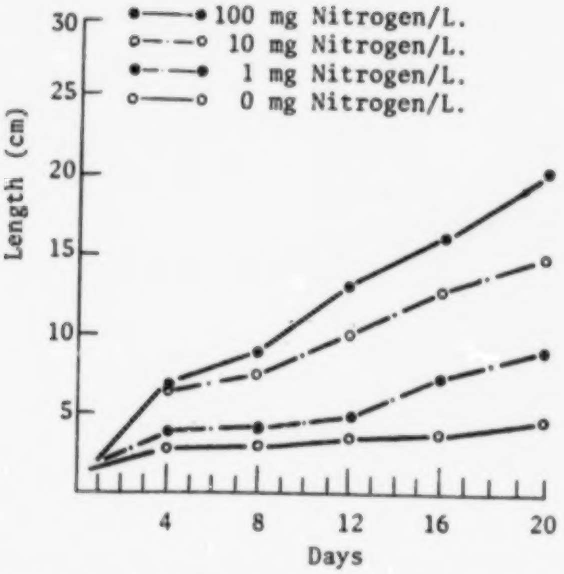


Figure 9. Increase in length of tips of Enteromorpha compressa placed in media containing increasing amounts of nitrogen. Temperature 20°C. Illumination 400 ft. C.

Trace Metals

Codium is rather unique in that it requires very low levels of metals. Original experiments with Codium were rather depressing because of the tendency of Codium to grow as an undifferentiated filamentous mass when placed in enriched sea water. During experiments it was noticed that best growth always occurred in aliquots without any enrichments. Further experiments showed that when only the trace metal solution was omitted, growth was even better than when all enrichments were omitted (Figure 10). At this point, we have not yet determined which metals are responsible for decreased growth.

Experiments with Enteromorpha compressa have shown that the trace metals presented in the TM-6 trace metal solution are stimulatory when presented as a mixed solution (Figure 11). In one experiment, copper increased growth up to about 300 μg per liter. The stimulatory effect of other metals has not yet been determined.

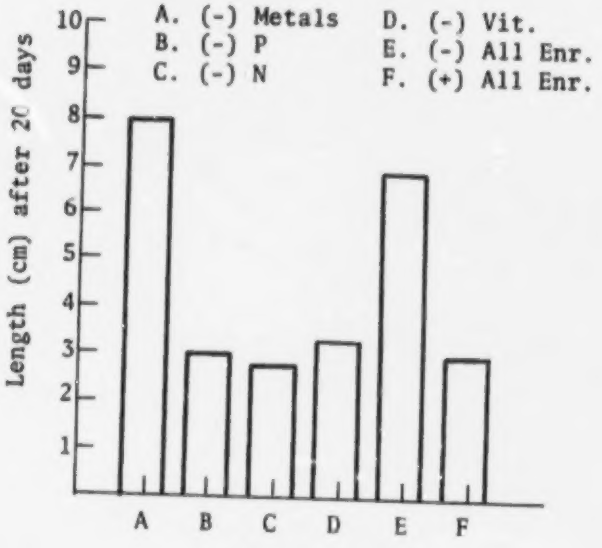


Figure 10. Increase in length of tips of Codium fragile placed in media deficient in various growth factors. Temperature 20°C. Illumination 400 ft. C.

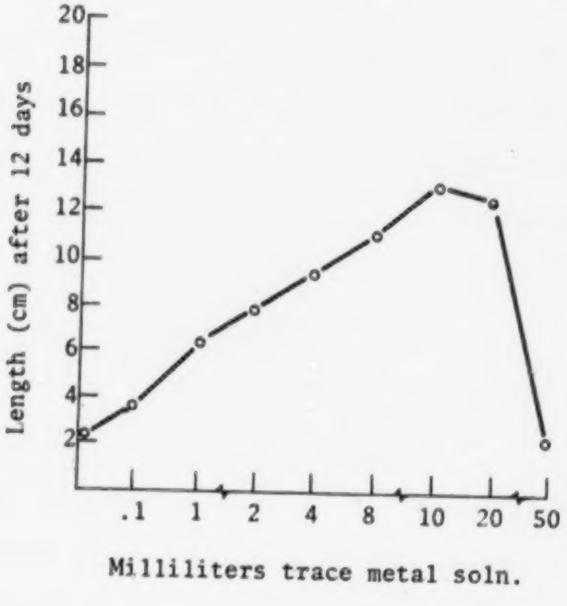


Figure 11. Increase in length of tips of Enteromorpha compressa when placed in increasing amounts of trace metal solution.

MISCELLANEOUS OBSERVATIONS

During the month of August 1972, large, round, non-flagellated spores were released from some tips of Codium and grew when placed in unenriched sea water. However, the individual spores developed into undifferentiated filamentous masses identical to tips grown in a complete, highly enriched sea water. These plants were retained for three months in culture but did not develop into mature Codium plants. Another interesting observation is the ability of Codium to grow from either end of an excised tip. This does not always occur, and the controlling factor for this phenomenon is not yet known.

DISCUSSION

The two species of algae, C. fragile and E. compressa, both green algae, both estuarine, and both abundant in nature, exhibit quite different responses to nutrients when studied in the laboratory. Codium appears to be a species with fairly limited salinity tolerances, and fairly limited temperature tolerances. Whereas E. compressaappears to be a species in which growth is definitely stimulated in highly enriched conditions, C. fragile does not appear to be stimulated by these conditions. It tends to prefer adequate but lower levels of enrichments.

The most outstanding difference in growth requirements of these two species is the requirement by C. fragile of a very low level of heavy metals. When heavy metal ions are abundant, growth is definitely depressed or non-existent.

How then do these two species co-exist in nature? To some extent, they are usually not found growing abundantly in the same places. Enteromorpha compressa and other Ulvales are abundant in bays and harbors that contain a high degree of organic pollution and raw sewage. Codium fragile is found in "salt ponds" or shallow embayments receiving a constant tidal flux. Could this type of environment tie up or chelate the metal ions, thereby allowing Codium to grow abundantly?

According to Fralick and Mathieson (1973) C. fragile photosynthesizes most efficiently at 20-25°C and at 900 ft. C. In our study however, growth, or increase in tip length was more efficient at light intensities of from 200-600 ft. C. Their optimum temperature compares well with ours, however. Studies by Borden and Stein (1969) on the Pacific coast of North America indicate that this optimum temperature for C. fragile from that coast is much lower (8°C).

The problem of differentiation of tissue into the dichotomies characteristic of Codium is not yet fully answered. Ramus (1972) obtained differentiation of the tissue by shaking. Borden and Stein (1969) did not obtain any differentiation of tissue. In my cultures, excized tips differentiate only in unshaken media with low metals. Cultures obtained from spores did not differentiate in this medium, however. Is it possible that growth requirements change as the thallus differentiates from juvenile filaments to the adult plant? Perhaps differentiation of all stages of Codium is dependent not only on concentration of heavy metals in the medium but on the osmotic pressures set up in the interstitial areas of the filaments of the plants. In an uncompacted juvenile plant this would be less important than in a tightly compacted adult thallus.

Enteromorpha compressa, rather than developing into a useful bioassay tool, is a good indicator of highly enriched water. In some instances, growth rates of 3-5 cm per day were obtained, and in a single low salinity experiment where nutrients were never limited over a six week period, a plant 1.5 m was obtained. With growth rates of this order, it is not difficult to understand how areas such as Boston Harbor and other highly enriched areas become choked with ulvaceous growth.

REFERENCES

Borden, C. and J. Stein. 1969. Mitosis and Mitotic Activity in *Codium fragile* (Suringar) Hariot. Phycologia 8(3): 149-156.

Davey, E., J. Gentile, S. Erickson and P. Betzer. 1970. Removal of Trace Metals from Marine Culture Media. Limnol. and Oceanogr 15(3):486-488.

Fralick, R. and A. Mathieson. 1973. Ecological Studies of *Codium fragile* in New England, USA. Marine Biology 19(2):127-132.

Guillard, R. and J. Ryther. 1962. Studies on Marine Planktonic Diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve) Gran. Can. J. of Micro. 8:229-239.

Levring, T., H. Hoppe, and O. Schmid. 1969. Marine Algae: A Survey of Research and Utilization. Botanica Marina Handbooks, Vol. 1. Cram, De Gruyter & Co., Hamburg.

Lewin, J. 1966. Silicon Metabolism in Diatoms. V. Germanium Dioxide, a Specific Inhibitor of Diatom Growth. Phycologia 6(1):1-12.

Moeller, H. 1969. Ecology and Life History of *Codium fragile* subsp. *tomentosoides*. Ph.D. Thesis. Rutgers University, New Brunswick, N. J. 76 p.

Perkins, E. and O. Abbot. 1972. Nutrient Enrichment and Sand Flat Fauna. Marine Pollution Bull. 3(5):70-72.

Ramus, J. 1971. *Codium*: The Invader. Discovery (New Haven, USA) 6:59-68.

__________. 1972. Differentiation of the Green Alga *Codium fragile*. Amer. J. Bot. 59:478-482.

GREAT LAKES NUTRIENT ASSESSMENT

Nelson A. Thomas, Katherine Hartwell,
and William E. Miller

INTRODUCTION

The large lakes program of the Grosse Ile Laboratory has both extramural and inhouse programs concerned with biostimulation. The extramural program deals with three different areas of algal growth studies. The first study is centered around the delineation of the nutrient requirement for the green alga Cladophora. This alga often causes nuisances around the Great Lakes during late summer when it breaks loose and washes up in windrows along swimming beaches. A basic knowledge of the nutrition and key growth factors is required in developing a sound basis for control of this alga. The first phase of this project is to develop culture techniques and synthetic culture medium. Secondly, the organic and inorganic nutritional requirements will be determined. A major part of the experiment will be to determine the critical concentration for each of the essential inorganic nutrients, thus hoping to determine the limiting nutrient concentration in the lake for Cladophora. Another phase of this project will be to sample the Cladophora growths in the lake and determine which element is restricting its growth.

Two types of procedures will be employed to determine the critical limiting nutrients for controlling Cladophora growths. The first procedure involves the comparison of the inorganic analysis of the alga with the critical growth concentration of elements as obtained in laboratory studies. The second procedure involves the assay of Cladophora samples for nitrogen and phosphorus availability through measurements of phosphorus releases upon boiling and ammonia uptake in the dark.

The second extramural project is the testing for the long-term availability of the various forms of nitrogen and phosphorus present in water samples collected at selected seasons in the Lake Ontario Basin. This includes both tributary and main lake sampling. The study will include the determination of those fractions of the nutrients that stimulate or control alga nutrient growths through a combined program of chemical analysis and nutrient assays. The chemical composition of the water during alga growth can be monitored, therefore, deleting those nutrients that are available for long-term alga growth. The second phase of this project is to determine the effect of reducing the phosphorus content in a particular water sample by using alum or iron salts. During this study it was found that the results could not be reproduced when the phosphorus was readded. When phosphorus was readded some growth element evidently, had been removed with the alum or iron salts. When phosphorus was readded in its pure form, the resultant growth was not equal to that of the untreated samples.

The third extramural project is the study of algal assay procedures that might be appropriate for the Great Lakes. These tests will include both Selenastrum capricornutum and natural populations of algae. Another phase is to determine the effect of various nutrient levels on the level of standing crop and which, if any, nutrients are limiting. This procedure includes the measurement of growth with nutrient additions both as a spike and as a constant concentration. This experiment will utilize the technique of chemically analyzing each test chamber daily and then adding the amount of nutrients to its initial level. The rationale for such experimental design is based on nutrient studies in the large lakes, which indicate that the nutrient concentration reductions are very long term. To perform experiments that have nutrient reductions over a ten day period is, therefore, unrealistic.

The inhouse program at the Grosse Ile Laboratory was designed to provide answers to the questions of the importance of phosphorus in limiting phytoplankton growth in Lake Michigan, as well as to attempt to define a phosphorus standard for Lake Michigan.

The subject of algal nutrient stimulation on the phytoplankton of Lake Michigan has been discussed in many reports including the Lake Michigan Enforcement Conference and the scientific literature. There has been strong evidence presented by Schelske and Roth (1973) that phosphorus has the greatest nutrient limiting effect on phytoplankton growth. The study on the relationship of the growth of phytoplankton to individual nutrients in relationship to Lake Michigan water is very incomplete. However, nutrient growth data are required to formulate criteria for the establishment of phosphorus limits in large bodies of water.

Algal assays were conducted on Lake Michigan water in connection with the Phosphorus Technical Committee to the Lake Michigan Enforcement Conference. Water samples were collected from a 3 meter depth along a transect from Milwaukee, Wisconsin, to Ludington, Michigan, during January, May, and Aug., 1972 (Figure 1). A similar transect was sampled between Benton Harbor, Michigan, and Chicago, Illinois. Samples were collected at the 6 meter depth during the months of April and July, 1972. A total of 25 samples were analyzed. The January, April and May samples analyzed by the National Eutrophication Research Program were assayed for growth potential utilizing the standard algal assay procedure with a Coulter Counter. Samples analyzed at the Grosse Ile Laboratory were assayed according to standard procedures with a fluorometer. In addition, the growth response of the natural population was also determined on selected split samples. The natural population growth was

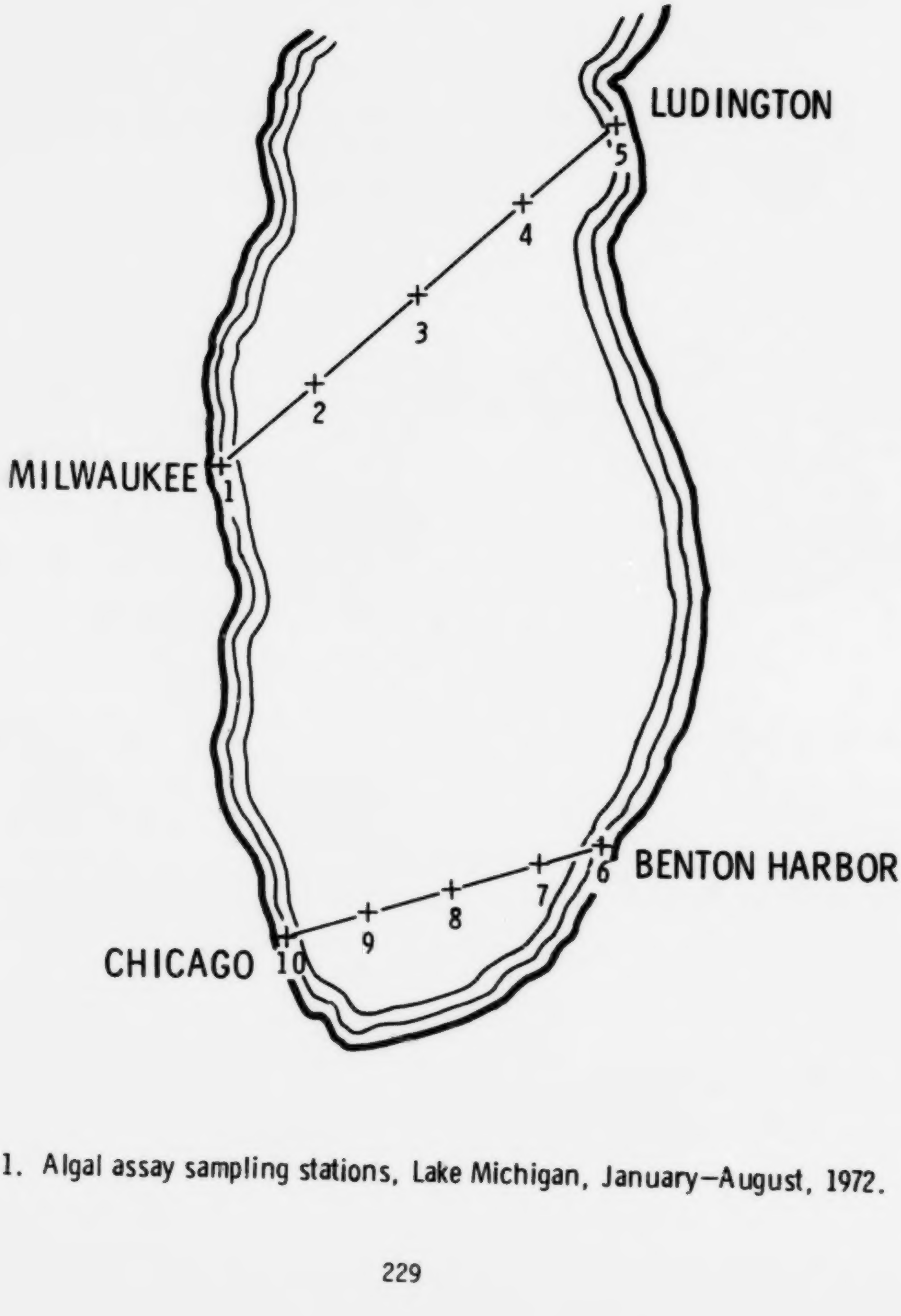

LUDINGTON
5
4
3
2
MILWAUKEE
1
BENTON HARBOR
6
7
8
9
CHICAGO
10

Fig. 1. Algal assay sampling stations, Lake Michigan, January–August, 1972.

estimated by the amount of chlorophyll a present in the samples. Raw water chemistry is presented in Table 1.

The samples analyzed according to the standard algal assay procedure received the following treatment:

Prior to assay each sample was pretreated in two ways: (1) one-half was filtered through a 0.45 micron membrane filter to remove indigenous biomass and (2) the other half was autoclaved to solubilize any nutrients contained within the indigenous biomass--equilibrated with a mixture of 1 percent CO_2 in air until the original pH was attained--and then passed through a 0.45 micron membrane filter. The assays were carried out in 500 ml Erlenmeyer flasks containing 100 ml of total sample. The following additions of nitrogen (as $NaNO_3$), phosphorus (as K_2HPO_4), iron (as $FeCL_3$), and trace elements contained in the Algal Assay Procedure medium singly or in combination were made to the Lake Michigan water samples: 1.00 mg N/1, .005 to .020 mg P/1, .033 mg Fe/1, and complete AAP medium trace element mixture. The nutrient combinations assayed are listed in Table 2.

Growth of the natural population was estimated by measuring the concentration of chlorophyll after 22 days of incubation. Samples were filtered through GF/C filters which were dissolved in acetone. Filters were ground and allowed to steep for one hour then centrifuged. The optical density of the extract was determined spectrophotometrically.

RESULTS

To determine the suitability of Selenastrum capricornutum as a test alga for Lake Michigan, split samples were run with the natural population as well as Selenastrum capricornutum (Tables 3, 4 and 5). The algal response to nutrient additions were similar except for the 5ppb phosphorus spike (Figure 2). The natural

TABLE 1 Lake Michigan chemistry when collected mg/1 - 18' all sample

Station Number	Sample No.	Date Collected	Ammonia Nitrogen	Nitrite Nitrate Nitrogen	Total P	Ortho-P	Alka-linity	Total dis P		Temp	Depth	pH
1	8647	5/72	.011	.201	.008	.001	105	<.001				
2	8648	5/72	.014	.211	.005	.002	A86	.003				
3	8649	5/72	A.0015	.21	.003	< .001	110	A.0025				
4	8650	5/72	.01	.21	< .003	.003	78	.001				
5	8651	5/72	< .01	.21	.004	< .003	127					
1	8908	8/72	.01	.15	.007	.006	119					
2	8909	8/72	.01	.14	.004	.003	118					
3	8910	8/72	.02	.15	.005	.003	116					
4	8911	8/72	.03	.15	.004	.003	116					
5	8912	8/72	.01	.16	.004	.003	115					
6	220	7/72	.02	.17	.011	.003	98			22.2	18'	8.6
7	223	7/72	.02	.15	.007	.003	114			23.0	18'	8.7
8	224	7/72	.02	.11	.006	.003	114			23.8	18'	8.6
9	225	7/72	.02	.11	.005	.003	114			19.5	18'	8.5

TABLE 1 Lake Michigan Chemistry when collected mg/1 - 18' all sample (Con't)

Station Number	Sample No.	Date Collected	Ammonia Nitrogen	Nitrite Nitrate Nitrogen	Total P	Ortho-P	Alka-linity	Total dis P		Temp	Depth	pH
10	228	7/72	.02	.13	.003	.003	118			16°	18'	8.4
6	8627	4/23/72	<0.01	.23	.006	<.003	131					
7	8626	4/23/72	<0.01	.22	<.003	< .003	160					
8	8625	4/23/72	<0.01	.21	.004	.003	133					
9	8624	4/23/72	<0.01	.21	.005	.003	129					
10	8623	4/23/72	<0.01	.19	.010	.008	129					
1	8482	1/11/72	.03	.28	.014	.003	136					
2	8483	1/11/72	.02	.26	.008	.003	144					
3	8484	1/11/72	.02	.26	.009	.007	138					
4	8485	1/12/72	.02	.25	.008	.004	141					
5	8486	1/12/72	.04	.28	.012	.004	138					

TABLE 2. EXPERIMENTAL DESIGN TO STUDY ALGAL GROWTH POTENTIAL OF LAKE MICHIGAN WATERS

Treatment

1. Lake water control
2. Lake water + .020 mg P/l
3. Lake water + 1.000 mg N/l
4. Lake water + 1.000 mg N/l + .020 mg P/l
5. Lake water + 1.000 mg N/l + .020 mg P/l + .033 mg Fe/l
6. Lake water + .020 mg P/l + .033 mg Fe/l
7. Lake water + .005 mg P/l + 1.000 mg N/l + .033 mg Fe/l + T.E.
8. Lake water + .010 mg P/l + 1.000 mg N/l + .033 mg Fe/l + T.E.
9. Lake water + .015 mg P/l + 1.000 mg N/l + .033 mg Fe/l + T.E.
10. Lake water + .020 mg P.l + 1.000 mg N/l + .033 mg Fe/l + T.E.

11. Full strength AAP medium

All samples were pretreated by (1) filtration, and (2) selected samples by autoclaving followed by filtration

TABLE 3 Corvallis Results. Maximum Standing Crop. Mg/1. Coulter Counter.

Station Number	Sample Number	Date Collected	Control	.02 P	1.0 N	.02 P 1.0 N	.02 P 1.0 N .033 Fe	.02 P .033 Fe	.005 P +all	.01 P +all	.015 P +all	.02 P +.all
1	8482 a & f M - 3	1/72	.066	.178	.065	3.762	.910					
2	8483 a & f M - ¼	1/72	.079	5.453	.046	2.873	1.558					
3	8484 a & f M - ½	1/72	.087	.505	.107	2.943	4.167					
4	8485 a & f M - 3/4	1/72	.133	.204	.034	.151	6.749					
5	8486 a & f L - 3	1/72	.103	.097	.028	1.379	4.651					
10	8623 a & f	4/72	.08	5.60	.12	8.88	9.40	7.32	.78	1.99	3.59	4.32
8	8625 a & f	4/72	.040	1.926	.060	4.422	4.444	5.181	.381	.722	1.520	2.085
9	8624 a & f	4/72	.189	6.257	.033	8.167	13.784	5.887	.307	.120	.370	.755
7	8626 a & f	4/72	.040	5.069	.046	6.921	2.776	5.784	.300	.865	1.493	4.031
6	8627 a & f	4/72	.050	5.764	.096	5.887	5.397	5.191	.825	.661	1.577	5.102
1	8647 a & f	5/72	.067	4.642	.061	6.51	6.80	5.55	.34	1.09	1.52	3.42
2	8648 a & f	5/72	.083	2.389	.056	1.121	2.061	1.569	.184	.379	1.220	.845
3	8649 a & f	5/72	.086	3.234	.069	4.296	9.017	5.244	.283	.519	1.117	1.355
4	8650 a & f	5/72	.115	3.723	.056	4.894	6.650	5.602	.136	.276	1.532	2.745

TABLE 3 Corvallis Results. Maximum Standing Crop. Mg/l. Coulter Counter. (Con't)

Station Number	Sample Number	Date Collected	Control	.02 P	1.0 N	.02 P 1.0 N	.02 P 1.0 N .033 Fe	.02 P .033 Fe	.005 P +all	.01 P +all	.015 P +all	.02 P + all
1	M-3 filtered	1/72	.193	3.858	.115	5.049	8.605					
2	M-1/4 f	1/72	.075	.059	.074	6.057	5.324					
3	M-1/2 f	1/72	.091	.299	.115	3.059	3.531					
4	M-3/4 f	1/72	.139	5.421	.043	.541	6.544					
5	L-3 f	1/72	.115	..038	.073	1.992	5.369					
10	8623 f	4/72	.04	5.52	.12	8.23	8.86	5.65	.14	2.12	3.13	4.69
2	8624 f	4/72	.067	1.996	.038	1.893	8.635	6.614	.067	.076	1.539	.149
8	8625 f	4/72	.036	.664	.045	1.811	1.646	.857	.575	.696	1.596	1.220
4	8626 f	4/72	.084	2.049	.049	1.178	.954	.573	.275	1.010	2.903	1.611
6	8627 f	4/72	.077	.216	.033	.193	.357	.551	.172	.802	1.011	.914
1	8647 f	5/72	.036	..754	.077	3.505	4.716	2.183	.838	1.527	1.734	2.225
2	8648 f	5/72	.085	3.222	.076	5.390	5.715	5.133	.449	1.162	2.116	2.085
3	8649 f	5/72	.059	4.552	.046	6.050	4.194	5.048	.717	.815	1.380	3.074
4	8650 f	5/72	.036	4.366	.058	5.060	8.850	5.457	.422	.991	2.035	2.969

TABLE 3 Corvallis Results. Maximum Standing Crop. Mg/l. Coulter Counter. (Con't)

Station Number	Sample Number	Date Collected	Control	.02 P	1.0 N	.02 P 1.0 N	.02 P 1.0 N .033 Fe	.02 P .033 Fe	.005 P +all	.01 P +all	.015 P +all	.02 P + all
5	8651 f	5/72	.055	3.210	.063	4.939	6.446	5.236	.463	2.103	2.460	4.648
5	8651 a&f	5/72	.067	2.480	.060	3.171	5.129	6.425	.178	.978	1.930	2.330

TABLE 4 Grosse Ile Calculated mg/m^3 Chlorophyll a Natural Algae. Spectrophotometer.

Station Number	Sample Number	Date Collected	Control	.02 P	1.0 N	.02 P 1.0 N	.02 P 1.0 N .033 Fe	.02 P .033 Fe	.005 P +all	.01 P +all	.015 P +all	.02 P + all
6	220	7/23/72	19	58.5	19	156	148	29.0	16.7	49.5	NA	119.5
10	228	7/23/72	107.5	76	91	296	214	37	111.5	177	NA	304.5
1	8908	8/16/72	9.8	7.6	7.2	37.9	NA	NA	NA	NA	NA	NA
5	8912	8/16/72	15.0	11.8	13.0	45.3	NA	NA	NA	NA	NA	NA

TABLE 5 Grosse Ile. Day 14 Standing Crop. Mg/l. Fluorometer

Station Number	Sample Number	Date Collected	Control	.02 P	1.0 N	.02 P 1.0 N	.02 P 1.0 N .033 Fe	.02 P .033 Fe	.005 P +all	.01 P +all	.015 P +all	.02 P +all
1	8647a,f	5/9/72	.002	8.36	.007	4.72	4.58	5.93	1.2	6.38	11.65	16.01
2	8648a,f	5/9/72	.011	6.657	.006	2.738	1.16	1.34	.187	6.17	9.26	10.61
3	8649a,f	5/9/72	0	1.6	0	.56	.49	3.4	1.6	6.5	11.6	16.3
3	8649f	5/9/72	.005	.47	0	.19	.28	.44	1.9	6.8	8.0	10.1
6	220f	7/23/72	.005	1.57	0	1.23	1.87	1.62	1.31	6.17	8.94	10.68
7	223f	7/23/72	0	.57	0	.22	.18	1.01	3.64	7.14	9.26	11.54
8	224f	7/23/72	0	.73	.011	.99	2.24	2.98	1.90	7.04	10.92	13.62
9	225f	7/23/72	.03	1.05	0	.68	.69	6.07	2.35	6.45	12.38	14.14
10	228f	7/23/72	0	4.61	.014	4.57	8.04	3.10	3.01	7.52	10.19	13.52
1	8908f	8/16/72	.019	1.17	.01	1.86	.98	2.45	.83	2.37	9.88	15.3
2	8909	8/16/72	.006	1.08	.007	1.02	.91	1.11	1.54	6.70	8.11	10.50
3	8910f	8/16/72	.037	1.41	.018	1.15	1.3	1.51	1.65	7.28	8.94	12.69
4	8911f	8/16/72	.028	1.91	.018	4.96	3.24	1.51	2.25	5.37	13.8	15.0
5	8912f	8/16/72	0	.08	0	4.37	.22	.26	.64	4.09	8.32	9.98

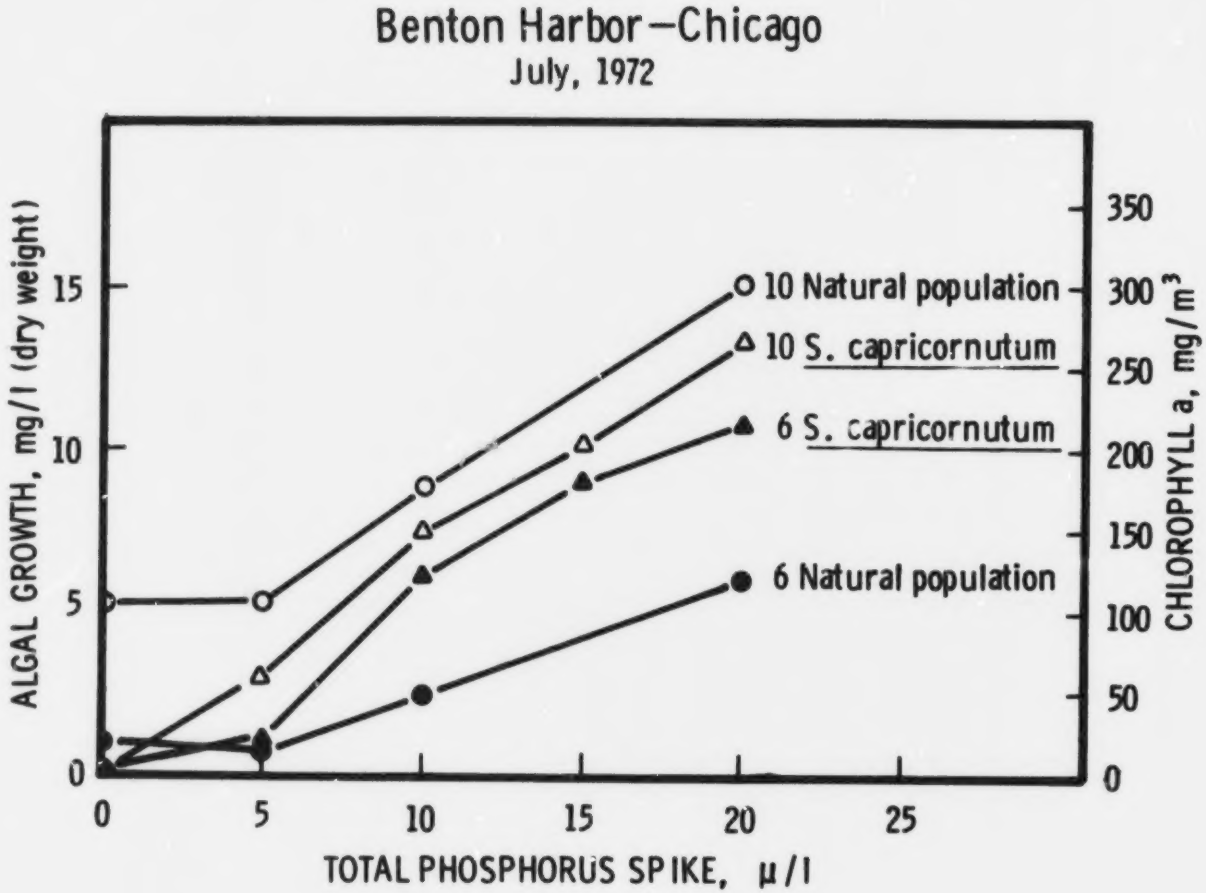

Benton Harbor—Chicago
July, 1972

Fig. 2. Growth of natural algae and Selenastrum capricornutum at various phosphorus concentrations.

population did not respond to the low level phosphorus spikes; the phosphorus content of the water was less than the critical level necessary to support growth of the natural population. The similarity of growth responses provides validity for the use of Selenastrum capricornutum to determine the limiting nutrients in Lake Michigan.

During January, all samples indicated phosphorus limitations by the increasing growth with increases in phosphorus concentration. The additional response to nitrogen indicates that nitrogen was secondarily limiting.

The April sampling of the transect from Benton Harbor to Chicago indicates that algal nutrient limiting effect increased from Benton Harbor to Chicago. The phytoplankton near Chicago (Station 10) had the greatest response to the addition of phosphorus and nitrogen thus indicating the supply of other nutrients and trace elements was sufficient. The autoclaved samples produced increased growth over the filtered samples thus indicating that autoclaving released additional nutrients. Phosphorus was also limiting in the autoclaved samples from April.

The results of the May sampling of the transect from Milwaukee to Ludington indicated the same phosphorus limitation. There was no response to the addition of nitrogen. However, the combination of nitrogen and phosphorus did produce a slightly higher growth than the single addition of phosphorus.

The response of the natural population to nutrient additions indicates that during July the near-shore water off Benton Harbor (Station 6) was limited primarily by phosphorus. Water samples collected near Chicago (Station 10) were primarily limited by phosphorus and secondarily by nitrogen. The near-shore waters were more nutrient limited than the off-shore stations.

During August the near-shore waters off Milwaukee were limited by phosphorus, secondarily by nitrogen. The near-shore water near Ludington was limited equally by phosphorus and nitrogen (Figure 3). The addition of trace elements was necessary to stimulate maximum growth.

The growth rate curve resulting from the addition of phosphorus to either Selenastrum capricornutum or to the natural population was linear. The increase in the population was directly proportionate to the concentration of phosphorus. The addition of other trace elements was requrired to stimulate the growth of Selenastrum capricornutum. In a few tests nitrogen was required in addition to phosphorus to cause a growth in the natural population.

The data was too limited to correlate the concentration of chlorophyll a and total phosphorus present at the time of the sampling. Likewise, the data points were too limited to correlate the chl a and dry weight (fluorometer) produced without spiking.

During January, the total and orthophosphate averaged 10.2 and 4.2 ppb, respectively. During May and August the same stations averaged 4.7 and 3.0 ppb for total and ortho phosphorus, respectively.

CONCLUSION

Algal nutrient limitations for Lake Michigan can be determined through the use of test algae (Selenastrum capricornutum) or with natural population.

Phosphorus is the primary limiting nutrient during January, April, May, July and August in the areas sampled. Nitrogen is secondarily limiting and in one case it was equal to phosphorus in limiting algal growth. Autoclaving of samples releases nutrients which results in additional growth. Tests in the laboratory indicate that on spiked samples there was a 5.0 percent increase in algal dry weight per increase in each ppb of phosphorus. In laboratory growth experiments with unspiked lake water, there is a 0.5

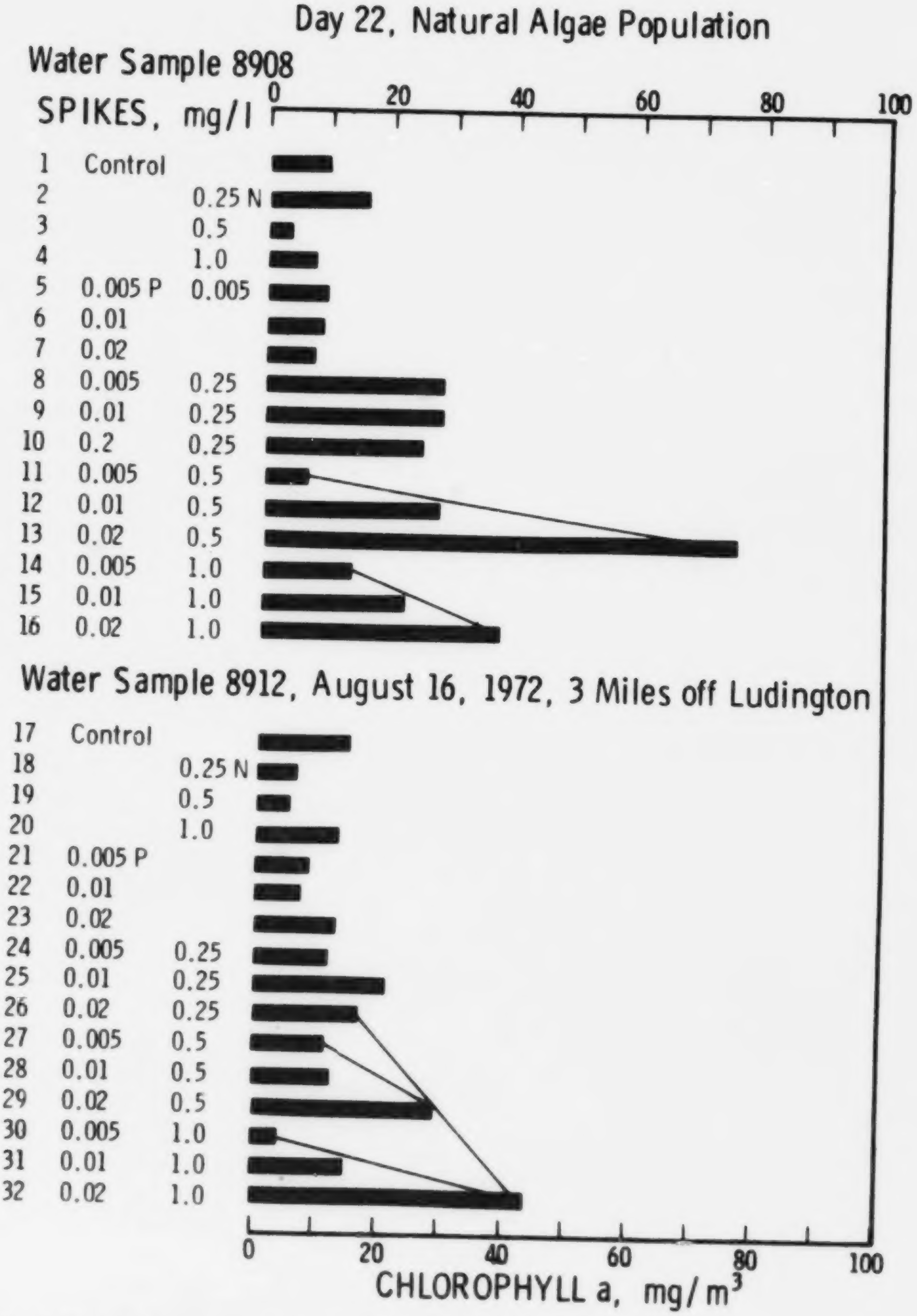


Fig. 3. Resultant growth of natural populations at various levels of nitrogen and phosphorus.

percent increase per ppb of total phosphorus. The difference in growth rates probably was caused by the unavailability of the phosphorus in the unspiked samples.

Lake Michigan, as indicated by the algal assay tests, is primarily limited by phosphorus and secondarily by nitrogen. In that Lake Michigan produces such large amounts of algae with small additions of phosphorus, a program to assess the present levels and interactions of phosphorus and phytoplankton must be implemented.

The results of these tests indicate that phosphorus limits the total algal standing crop in Lake Michigan. The results also indicate that changes in the ppb range substantially changes the standing crop; therefore, to base a phosphorus standard on the tests thus far performed, is not possible. Algal assay work is being continued in an effort to establish a standard.

REFERENCE

Schelske and Roth. 1973. Limnological Survey of Lakes Michigan, Superior, Huron and Erie. University of Michigan, Great Lakes Research Division, Publication No. 17. pp 108.

ASSESSING TREATMENT PROCESS EFFICIENCY WITH THE ALGAL ASSAY TEST

Richard E. Thomas and Robert L. Smith

INTRODUCTION

Wastewater discharges containing substantial quantities of nitrogen and phosphorus have been identified as an important factor in the eutrophication problem plaguing many lakes throughout the United States. The development of a reliable algal assay procedure for assessing the sensitivity of receiving waters to change and the effects of changes in waste treatment processes on receiving waters offers the hope of extending the assay procedure to other applications.

Ongoing research studies on the treatment efficiency of the spray-runoff (overland flow) method of wastewater treatment by land application provided us an opportunity to make preliminary evaluations for one such extension of the algal assay procedure. One of these ongoing research studies involved spray-runoff treatment of rainfall runoff from a beef cattle feedlot. The other ongoing research study has the primary objective of determining the feasibility of utilizing the spray-runoff soil treatment process as a simple and economical system for complete treatment of raw domestic wastewater. The algal assay procedure was included in the analytical program for these two studies to obtain a measure of the capability of spray-runoff to reduce the potential of a wastewater to stimulate algal growth. The primary objective of including the algal assay procedure in the analytical program was to observe the relative reduction in the capability to stimulate algal growth in relation to the reduction in nitrogen and phosphorus concentrations achieved by the treatment process.

METHODS

All samples were collected in non-metallic containers and either transported to the analytical laboratory in a few minutes or packed in an ice chest for transport to the analytical laboratory. Indigenous algae were killed by autoclaving rather than removed by filtration since it was desired to determine the algal biomass that could be grown from all nutrients in the water including those solubilized by autoclaving. The standard bottle test procedure was followed utilizing *Selenastrum capricornutum* as the inoculum and measuring the development of the maximum standing crop with fluorescence measurements and a gravimetric determination at the end of the test period. Aliquots of each sample were analyzed for solids, biochemical oxygen demand, total nitrogen, and total phosphorus as well as many other parameters of interest for evaluating treatment efficiency of the spray-runoff treatment process.

RESULTS

Two algal assay tests have been completed for each of the ongoing research projects. Samples for the tests involving the runoff from the beef cattle feedlot were taken from a storage lagoon from which the wastewater was pumped to the spray-runoff area and from the spillway of a small farm pond which was the final step in the multi-unit treatment system. Samples for the tests involving the municipal wastewater were taken from the discharge line of the standard rate trickling filter plant used by the city and from the overflow at a flow measuring flume at the spray-runoff test area. The results of the algal assay tests and pertinent chemical quality data are summarized in Table 1.

Table 1. CHEMICAL QUALITY AND BIOMASS PRODUCTION (mg/l)

Test Liquid	Total Suspended Solids	Biochemical Oxygen Demand	Total Nitrogen	Total Phosphorus	*Calculated Biomass Production
		Feedlot Runoff			
Storage lagoon	81	6	15.2	3.7	220
	127	25	11.5	4.6	194
Farm Pond	11	5	3.6	0.2	31
	8	6	6.3	0.5	53
		Municipal Wastewater			
Sewage Plant Effluent	32	13	20.8	8.5	198
	20	16	16.3	7.3	121
Spray-Runoff Plots	20	20	6.4	2.5	90
	10	10	4.0	1.0	60

*Biomass calculated with factor derived between fluorescence measurements and gravimetric measurements for feedlot tests.

The treatment system consisting of the spray-runoff area and the farm pond achieved substantial reductions in the concentration of all four of the chemical parameters shown in Table 1 for the studies on the feedlot runoff. Changes observed in biomass production paralleled the changes in the total nitrogen and total phosphorus concentrations but showed little relationship to the total suspended solids or biochemical oxygen demand.

The spray-runoff treatment system for municipal wastewater produced a product water of better chemical quality than the standard rate trickling filter plant. As was the case for the feedlot test, the biomass productivity also paralleled the changes in total nitrogen and total phosphorus.

DISCUSSION

The results of two algal assay tests conducted on each of two wastewater treatment systems support the hypothesis that the algal assay test shows promise for evaluating the eutrophying potential of product waters from different wastewater treatment processes. Even though nitrogen and phosphorus concentrations of the product waters were substantially greater than those in waters for which the algal assay test was developed, biomass production was directly related to the concentration of nitrogen and/or phosphorus in the product water. The general relationships observed in these two tests appear suitable for direct comparisons at a specific location. The different relationships observed for the feedlot tests versus the municipal wastewater tests indicate that attempts to extrapolate results between wastewaters and to differing wastewater sources could lead to erroneous interpretations.

SUMMARY

The algal assay test was included in the evaluation of wastewater treatment processes for feedlot runoff and for municipal wastewater. The biomass productivity was directly related to the total nitrogen and/or phosphorus concentration in the product water from the treatment process. The results of these exploratory tests suggest that the algal assay test has promise for comparing the eutrophying potential of the effluents from a variety of wastewater treatment processes. This capability to measure eutrophying potential could be an important factor in determining cost effectiveness of treatment processes in many localities.

UTILIZATION OF ENERGY BY PRIMARY PRODUCERS IN FOUR PONDS IN NORTHWESTERN FLORIDA

Gerald E. Walsh

INTRODUCTION

Amounts of solar energy incorporated by primary producers in four small ponds in northwestern Florida were estimated by use of field data collected during an annual cycle and current theory of photosynthesis as discussed by Arnon et al. (1968), Kok (1965), and Rabinowitch and Govindjee (1969). Several reports have described photosynthetic rates in relation to light intensity (Ryther 1956, McAllister 1961, Steeman Nielsen and Hansen 1961, Goldman et al. 1963, Strickland 1965, Kalff 1969), but none has given the amount of solar energy incorporated by photosynthesis into organic compounds in natural communities.

The research reported here was concerned with seasonal changes in energy utilization. The objectives of the investigation were to:

1) determine the amount of solar energy incorporated into the first trophic level in each pond,

2) relate energy incorporated to the amount of solar radiation impinging upon the water,

3) determine the efficiency of incorporation of energy absorbed by primary producers into organic compounds,

4) study the role of CO_2 concentration in limiting the rate of primary production, and

5) describe changes in phytoplankton over a seasonal cycle.

DESCRIPTIONS OF THE PONDS

The ponds were located near Pensacola, Florida, at approximately 87°9' West Longitude and 30°20' North Latitude. Their bathymetric contours are given in Figure 1; area, volume, and depth in Table 1; and pH, nitrate-nitrogen, alkalinities, and conductivities in Table 2.

TABLE 1. AREA, VOLUME, AND DEPTH OF PONDS USED IN ENERGY STUDIES.

Pond	Area (ha)	Volume (m^3)	Avg. Depth (m)
1	1.95	17,800	0.91
2	2.30	16,200	0.71
3	0.91	9,600	1.06
4	0.15	650	0.43

Each pond had a narrow band of emergent vegetation (*Typha* sp.) around the margin, and each was surrounded by nearly bare sand. Substrata were coarse sand that contained increasing amounts of organic debris with depth. Water color was light brown, and a Secchi disc could be seen resting on the bottom at maximum depth in each basin on all sampling days. Thermal stratification was never observed.

The ponds appeared highly productive of vegetation. *Potamogeton pectinatus* and *Chara* sp. grew in all ponds. The phytoplankton was dominated by the blue-green algae *Lyngbya* sp., *Anabaena* sp., and *Oscillatoria* sp., and the green alga *Oedogonium* sp. The green alga

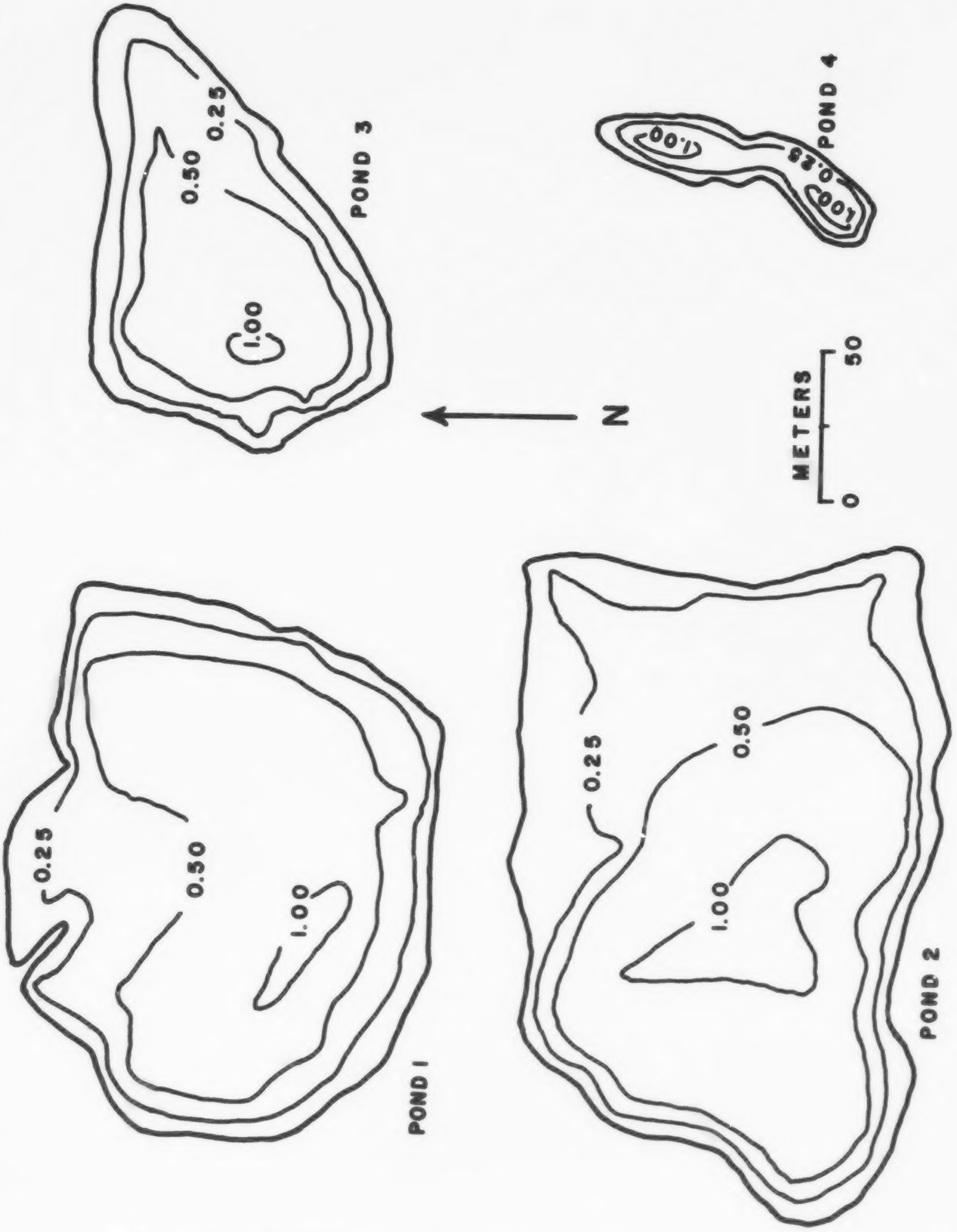

POND 3
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Figure 1. Bathymetric Contours of Ponds Used in Energy Studies

TABLE 2. AVERAGE pH, NITRATE-NITROGEN, ALKALINITY, AND CONDUCTIVITY IN PONDS, 1968.

	pH		NO_3, mg/1		Alkalinity	Conductivity
Pond	8 AM	2 PM	8 AM	2 PM	mg $CaCO_3$/1	millimhos
1	7.35	7.60	0.107	0.099	23.95	1.10
2	8.44	8.62	0.095	0.092	43.29	2.71
3	7.77	7.99	0.122	0.116	68.17	1.22
4	7.06	7.82	0.227	0.198	42.66	0.85

Spirogyra sp. was present between March and May, and *Zygnema* sp. was found in small numbers between January and May in Ponds 1, 2, and 3.

Unicellular algae, mostly diatoms, were never prominent in the phytoplankton. The most numerous genera were *Pinnularia*, *Tabellaria* *Navicula*, *Pleurostigma*, *Stauroneus*, *Amphora*, *Synedra*, *Diatoma*, and *Cymbella*.

METHODS AND THEORY

Water samples were taken from each basin with a plastic Van Dorn bottle at approximately weekly intervals from 22 January to 11 December 1968. Water was collected at approximately 0800 and 1300 hours at the shore, and at the surface, mid-depth, and bottom in the basins of Ponds 1, 2, and 3, and at the surface, mid-depth, and bottom of Pond 4 in both basins. Water was analyzed for dissolved oxygen by the Winkler method (Strickland and Parsons 1965). Gross oxygen production of the total pond was estimated by photosynthesis/respiration ratios (photosynthetic quotient, Q_p) for algal production. Photosynthetic quotients were calculated by the light-and-dark bottle method (Strickland and Parsons 1965), and bottles were incubated *in situ* at the depth of collection. Although photosynthetic quotients derived from algal productivity are not exactly those of the total pond, they are more likely to be better approximations than the constant values of about 1.0 used by some workers because they reflect seasonal changes. Corrections were made for diffusion of oxygen across the surface of the water (Welch 1968) and for oxygen stored in intracellular lacunae of rooted plants (Hartman and Brown 1967).

Total chlorophyll was determined by the method of Strickland and Parsons (1965). Free CO_2 was calculated from total alkalinity and pH values (Rainwater and Thatcher 1960). Uptake of CO_2 was calculated from its diurnal changes in the water (Verduin 1956). The data between 0800 and 1300 hours were plotted and integrated in relation to length of day to yield values for the total number of hours of sunlight.

Nitrate-nitrogen was measured by the method of Kahn and Brezenski (1967). Phytoplankton were counted each week. Samples were collected with a plankton net (155 μ porosity) towed beneath the water surface by a boat. The volume of water filtered was 7.2 cubic meters. Algae were counted in a Sedgwick-Rafter counting chamber.

Several diel studies were made in which water samples were taken at three-hour intervals from 0600 hours of one day to 0600 hours of the next. Chemical analyses were identical to those described above.

On 3 April 1968 a wettable powder formulation of the herbicide dichlobenil (Casoron®, 2,6-dichlorobenzonitrile) was applied at an active ingredient concentration of 1.0 ppm to Pond 4 in order to study fixation of solar energy by algae in the absence of rooted plants. Dichlobenil is effective in control of rooted plants but has no demonstrable effect upon unicellular or filamentous algae at the concentration used.

According to current theory of photosynthesis, there are two

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biochemical reactions that require light energy (the "light reactions") and one that does not require light (the "dark reaction"). Molecular oxygen is formed from the first light reaction:

$$2H_2O \xrightarrow{\text{light}} 2O_2 + 4H^+ + 4e^-, \qquad (1)$$

and reduced NADP is formed in the second light reaction:

$$2NADP + 4e^- + 2H^+ \xrightarrow{\text{light}} 2NADPH. \qquad (2)$$

In the dark reaction, energy from (1) and (2) is utilized to incorporate CO_2 into carbohydrate:

$$2H^+ + 2NADPH + CO_2 \longrightarrow 2NADP + H_2O + CH_2O. \qquad (3)$$

The amount of energy required for evolution of any observed amount of oxygen can be calculated. Eight to ten photons are required for evolution of one molecule of oxygen by the light reactions (Clayton 1965). I used nine photons in the following calculations.

Light, as it impinges upon the pigment system, is converted to red quanta at wavelengths between 680 and 700 nm (Kok 1965). The energy content of one Einstein (6.02×10^{23} quanta) of light at a wavelength of 700 nm is 41 kcal.

If the number of oxygen molecules evolved over a period of time is known, the rate of absorption of energy can be calculated. The example below, taken from Pond 4 on 4 April, shows how energy required for evolution of oxygen was calculated:

Oxygen evolved, molecules/M^2/day $= 1.91 \times 10^{23}$

Number of photons required/M^2/day $= 17.19 \times 10^{23}$

Number of Einsteins/M^2/day

$$= \frac{17.19 \times 10^{23}}{6.02 \times 10^{23}} = 2.86$$

Total energy absorbed = 2.86 X 41 kcal = 117 kcal/M^2/day.

Energy incorporated by light reactions is not transferred with 100 percent efficiency to organic compounds in the dark reaction. For the general reaction

$$H_2O + CO_2 \xrightarrow{\text{light}} \text{Organic molecules} + O_2$$

approximately 180 kcal of light energy are required to evolve one mole of oxygen (Kok 1965). Using the data from above, 0.317 moles of oxygen were evolved/M^2/day. If 180 kcal were incorporated into organic matter for each mole of oxygen evolved, then 57 kcal/M^2/day were stored and available for respiration and as food for herbivores. Hereafter, this energy, which is that potentially stored in organic molecules, is referred to as ε. Efficiency of ε may be calculated from the expression

$$\emptyset\varepsilon = \frac{\varepsilon}{\text{insolation}} \times 100.$$

Walsh and Heitmuller (1971) reported mean monthly insolation on the ponds and those data were used to calculate $\emptyset\varepsilon$.

Expressions (1), (2), and (3) imply that when substances such as inorganic phosphorus and nitrogen are limiting, solar energy can be utilized and oxygen evolved through conventional photosynthetic pathways without formation of organic molecules.

The limiting effect of CO_2 concentration upon primary production and the efficiency of conversion of ε to organic compounds was estimated. Pond 2 contained very little dissolved CO_2, Ponds 1 and 3 contained larger amounts, and Pond 4 contained the greatest concentration of free CO_2. Approximately 140 kcal of light energy are required for incorporation of one mole of CO_2 into organic molecules and the efficiency of storage is 81% (Bassham 1965). The amount of energy actually stored was estimated by assuming that all CO_2 absorbed was used to form new organic molecules. The number of moles of CO_2 absorbed, multiplied by 113 (0.81 X 140 kcal), gave an estimate of the amount of energy stored. This estimate, hereafter called E, must necessarily be a minimum value for gross primary production because utilization of endogenous CO_2 was not considered.

There is another important factor to consider here. The values of E are underestimations of productivity because the method used estimated free CO_2 in a system where

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^=$$

and excluded CO_2 in other forms, such as $CaCO_3$. This underestimation is especially significant for data from Pond 2, where the average annual alkalinity was 43.3 mg $CaCO_3$/1 but the pH was always high (e.g., 9.34 on 10 September).

The rate and efficiency with which light energy is used to form new compounds with CO_2 are two basic ecological processes associated with primary production. Efficiency was estimated from the expression

$$E' = \frac{E}{\varepsilon} \times 100$$

EFFECTS OF HERBICIDE

Dichlobenil at 1 part per million reduced the density of benthic plants in Pond 4. After application of the herbicide on 3 April, *P. pectinatus* and *Chara* sp. declined in numbers. Effects of the herbicide appeared greatest around 1 May, when all *P. pectinatus* and approximately 70 - 80% of the *Chara* sp. were eliminated. As benthic plants died, a bloom of filamentous algae occurred. In Pond 4 at the height of the bloom, dominant phytoplankters were *Lyngbya* sp. (533,400 filaments/M^3), *Oedogonium* sp. (327,150 filaments/M^3), and *Oscillatoria* sp. (35,520 filaments/M^3). In the other ponds, algae did not bloom at that time. Presumably, as benthic plants died, the algae utilized nutrients released from them to sustain the bloom. By 1 June, *Chara* sp. had returned to approximately the pretreatment density, a few individuals of *P. pectinatus* had appeared, and the algal bloom had subsided. By 2 July, density of *P. pectinatus* appeared similar to that existing before treatment. Treatment had no measured effect on energy incorporation.

ENERGY INCORPORATION AND UTILIZATION

Diel studies demonstrated differences between ponds in the amount of solar energy incorporated by the light reactions of photosynthesis, efficiency of its incorporation, the rate of uptake of CO_2, and the amount of light energy stored (Table 3). Pond 4 had the greatest primary productivity and absorbed the greatest number of einsteins and quantity of CO_2 per square meter. In addition, $\phi\epsilon$ values and the amount of energy stored (E) were much greater than in the other ponds. During a phytoplankton bloom in May, when most benthic plants were gone, total chlorophyll concentration in Pond 4 was 25.3 mg/ℓ and energy stored was 23.4 kcal/M^2/day. In Pond 3 on the same day, total chlorophyll content was only 3.9 mg/ℓ and energy stored was only 2.2 kcal/M^2/day.

Incorporation and utilization of energy in Pond 2 differed greatly from those in the other ponds. During the diel study, Pond 2 contained relatively small numbers of phytoplankters as shown by total chlorophyll concentration, but its bottom was covered by dense growth of *P. pectinatus*. Concentration of free CO_2 was 1.5 mg/ℓ at 0600 hours and 0.4 mg/ℓ at 1500 hours and was, therefore, limiting to photosynthesis. Rate of incorporation of solar energy by the light reactions of Pond 2 was relatively rapid because of the dense growth of benthic plants. Rate of incorporation into organic molecules was low however, probably due to the limiting effect of low concentration of CO_2. Although rate of absorption of energy in Pond 2 was more than double that in Pond 1, the estimated rate of absorption of CO_2 was only 1/6th that in Pond 1.

Values of E' are estimates of efficiency of incorporation of

TABLE 3. ENERGY OF LIGHT REACTIONS, AMOUNT OF CO_2 TAKEN UP, AND MAXIMUM AMOUNT OF LIGHT ENERGY STORED DURING DIEL STUDIES IN PONDS. (ε = solar energy potentially stored in organic molecules, $\phi\varepsilon$ = efficiency of ε, E = estimate of amount of energy stored from CO_2 uptake data, E' = ratio of E to ε X 100).

	30 Nov. - 1 Dec. 1967		7 - 8 Dec. 1967		28 - 29 Mar. 1968		2 - 3 May 1968	
	Pond 1	Pond 2	Pond 3	Pond 4	Pond 3	Pond 4	Pond 3	Pond 4
Einsteins/M^2/day	0.64	1.60	0.55	1.69	0.99	2.05	1.62	4.59
ε, kcal/M^2/day	14.40	36.00	12.40	38.00	22.30	46.10	36.50	103.30
$\phi\varepsilon$, %	0.52	1.30	0.65	1.98	0.79	1.64	0.96	2.72
CO_2, mm/M^2/day	27.50	4.70	23.10	116.60	15.70	96.30	19.40	206.60
E, kcal/M^2/day	3.10	0.50	2.60	13.20	1.80	10.90	2.20	23.40
Chlorophyll, mg/1	1.20	0.10	1.20	3.10	1.90	2.00	3.90	25.30
E', %	21.50	1.40	21.00	34.80	12.60	23.60	6.00	22.70
Q_p	1.02	0.56	0.95	0.54	0.72	0.91	1.22	1.76

trapped solar energy into organic compounds. Table 1 shows that Pond 4 was most efficient and Pond 2 was least efficient. Ponds 1 and 3 were approximately equal in their ability to transfer energy to organic molecules during diel studies in December.

ANNUAL STUDIES

Annual variations in the amount of energy required for production of the calculated amounts of oxygen are given in Figure 2. In all ponds, the amount of energy used in the light reactions was greatest in summer, and monthly average reached a peak of 11.1 Einsteins/M^2/day in Pond 4 in June. The amount of energy that could be stored in organic substances, assuming 100 per cent efficiency of transfer, is estimated by the value of ε. This varied between an average of 4.5 kcal/M^2/day in Pond 1 in February and an average of 250.5 kcal/M^2/day in Pond 4 in June. The primary producers in Pond 4 absorbed a greater amount of energy than did those in the other ponds. Extremes of average efficiency, expressed as $\phi\varepsilon$, were 0.1 per cent in Pond 3 in December and 7.2 per cent in Pond 4 in September. On an annual basis, Pond 4 was most efficient in the absorption of light energy (Table 4). The monthly averages of CO_2 absorbed and energy required for its fixation are given in Figure 3. In each pond, except Pond 2, there was an increase in the rate of uptake of CO_2 in summer. Calculated CO_2 concentrations in Pond 2 were very low throughout the summer. Between April and November, highest concentration was 0.30 mg/ℓ, compared to 16.9 mg/ℓ in Pond 4 in July (Table 5).

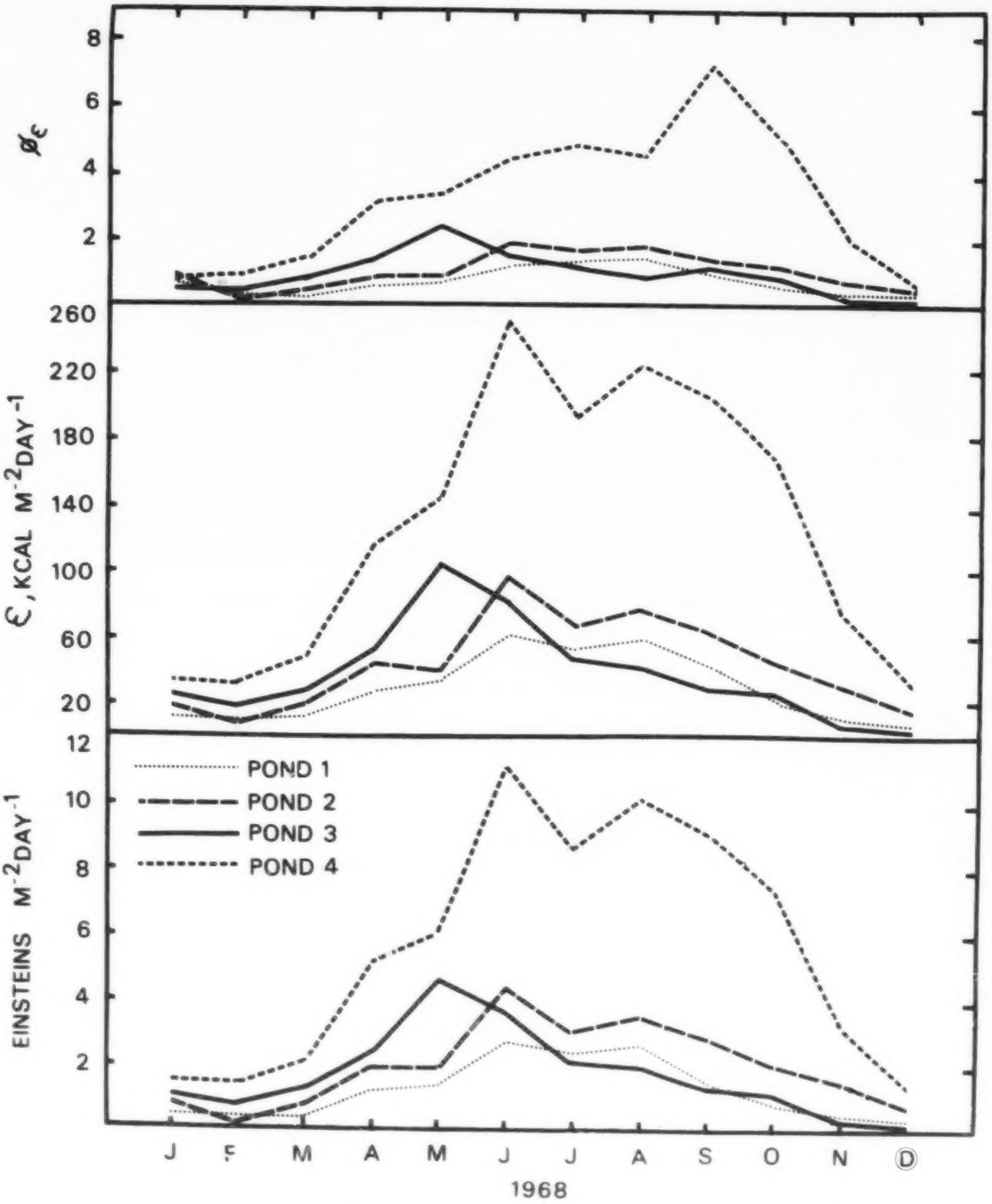


Figure 2. Mean Monthly Solar Energy Utilized by Light Reactions in Ponds, 1968.

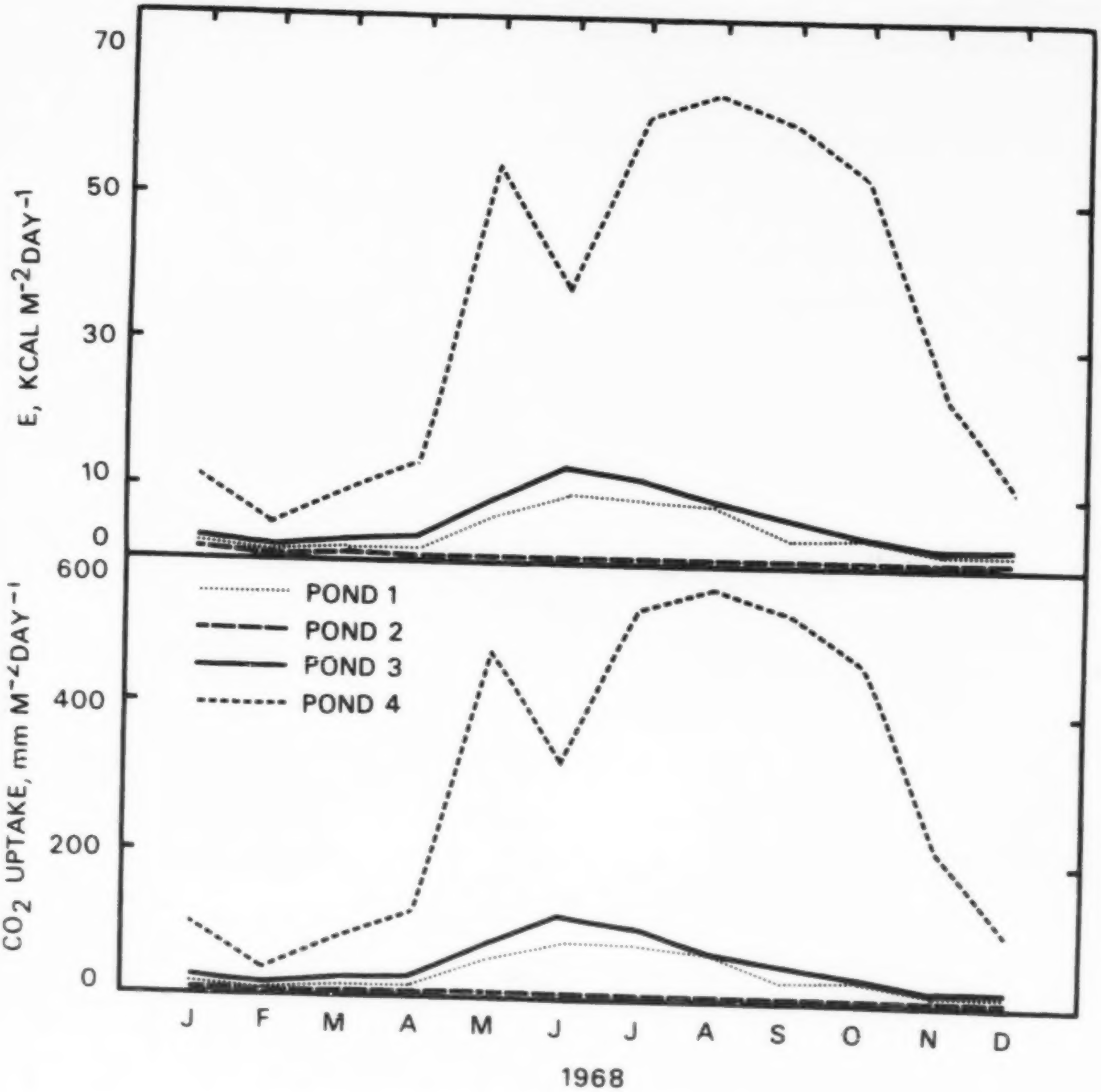


Figure 3. Mean Monthly Values of Rate of Uptake of CO_2 and Energy Required for its Fixation, 1968.

TABLE 4. MEAN ANNUAL EFFICIENCY OF ABSORPTION OF LIGHT ENERGY IN PONDS, 1968.

Pond	$\phi\epsilon$,%
1	0.7
2	1.0
3	0.9
4	3.1

TABLE 5. CONCENTRATIONS OF FREE CO_2 IN PONDS, 1968.

Pond	CO_2, mg/l	
	Annual average	Daily range
1	1.4	0.1 - 3.3
2	0.4	0.0 - 1.7
3	1.8	0.2 - 5.3
4	5.0	0.1 -16.9

Although measured concentrations of CO_2 were low in Pond 2, it should not be construed that productivity was low. From 4 June to 6 November, afternoon pH values were between 8.89 and 9.34 (average 9.11). Free CO_2 was seldom found in the water between those dates, whereas blue-green algae attained their highest numbers during that time. Clearly, a source of CO_2, other than CO_2 as we measured it, was utilized by primary producers in Pond 2. For discussions of chemical equilibria that relate to CO_2 in natural waters, see Schindler (1967) and Lee and Hoadley (1967).

If all CO_2 absorbed was incorporated into organic compounds (E), the amounts of energy fixed were greatest in Pond 4, where an average of 64.0 kcal/M^2/day was required in August. On a seasonal basis, energy required for fixation of CO_2 varied widely between ponds and within any one of them, but was greatest in Pond 4 (Table 6).

Efficiency of transfer of absorbed light energy to organic compounds was greatest in Pond 4 (Table 7). Pond 2 absorbed more light energy than Ponds 1 and 3, probably because of dense growth of _P. pectinatus_, but was less efficient in formation of new compounds due to low concentrations of free CO_2. At the concentrations of free CO_2 in Pond 2, photosynthesis was rate-limited because of its dependence upon bicarbonate as a source of carbon (Wright and Mills 1967, Fogg 1968). Thus, it seems probable that rates at which the light reactions could proceed were not limited, but photosynthesis was limited by the slow rate of supply of CO_2 to the dark reaction.

TABLE 6. ENERGY REQUIRED FOR FIXATION OF CO_2 IN PONDS, 1968.

Pond	E, kcal/M^2/day	
	Annual Mean	Daily range
1	4.0	1.1 - 10.4
2	0.8	0.5 - 5.8
3	5.4	0.1 - 14.9
4	33.5	2.8 - 89.9

TABLE 7. EFFICIENCY OF INCORPORATION OF ABSORBED ENERGY CALCULATED FROM CO_2 DATA (E'), 1968.

Pond	Annual average, %	Range, %
1	15.3	4.4 - 25.4
2	3.2	0.7 - 12.9
3	17.8	6.1 - 46.5
4	26.8	11.3 - 37.2

Great limitation did not occur in the other ponds, where concentrations of free CO_2 were not consistently low. Primary producers of Pond 4 absorbed the greatest amount of light energy and utilized the greatest amount of free CO_2. They were also most efficient in transfer of light energy to organic compounds, possibly because concentrations of free CO_2 were seldom limiting.

There was no demonstrable effect of herbicide on absorption of solar energy or uptake of CO_2 in Pond 4. Rate of absorption of solar energy increased in all ponds from March to June, and the increased rate of uptake of CO_2 in Pond 4 when benthic plants were almost gone was accompanied by increases in Ponds 1 and 3.

PHYTOPLANKTON RELATIONSHIPS

The average number of filamentous phytoplankters/M^3/day, average number of blue-green algae/M^3/day, and the percentage of blue-green algae in the total for each month are given in Tables 8, 9, 10, and 11. Blue-green algae bloomed in the summer to such degree that green algae were almost excluded from the ponds.

The photosynthetic quotients also increased during the summer and were as high as 2.7 during the blue-green algal blooms (Tables 8 - 11). Fogg (1968) stated that (1) the photosynthetic quotient for young, growing plants and algal cultures is approximately 1.1, such a value indicating that the primary product of photosynthesis is carbohydrate, and (2) the photosynthetic quotient for plant tissue or algal cells in a later phase of growth, where fat accumulation

TABLE 8. AVERAGE TOTAL NUMBER OF FILAMENTOUS PHYTOPLANKTERS AND AVERAGE NUMBER OF BLUE-GREEN ALGAE PER CUBIC METER ON EACH SAMPLING DAY, AND PHOTOSYNTHETIC QUOTIENT (Q_p) IN POND 1, 1968.

Month	Total Number	Blue-Green	% Blue-Green	Q_p
J	49,314	3,473	7.0	0.6
F	37,935	2,060	5.4	1.1
M	19,867	1,867	9.3	1.2
A	88,685	4,060	4.6	1.0
M	73,302	15,754	21.5	1.9
J	152,290	149,447	98.1	2.3
J				2.3
A	*Lyngbya* too numerous and matted to count			2.5
S				2.1
O	116,443	57,454	49.3	1.1
N	26,620	10,914	40.9	1.1
D	16,498	6,061	36.7	1.0

TABLE 9. AVERAGE TOTAL NUMBER OF FILAMENTOUS PHYTOPLANKTERS AND AVERAGE NUMBER OF BLUE-GREEN ALGAE PER CUBIC METER ON EACH SAMPLING DAY, AND PHOTOSYNTHETIC QUOTIENT (Q_p) IN POND 2, 1968.

Month	Total Number	Blue-Green	% Blue-Green	Q_p
J	58,819	17,381	29.6	0.9
F	70,388	7,524	10.7	0.6
M	55,710	5,309	9.5	0.5
A	189,528	17,600	0.9	0.3
M	761,580	213,532	28.0	0.9
J	*Lyngbya* too numerous and matted to count			1.2
J				1.4
A	111,980	105,670	94.4	1.8
S	15,088	13,700	90.8	1.2
O	17,119	16,599	97.0	1.1
N	3,120	2,210	70.8	1.0
D	7,930	4,680	59.0	0.4

TABLE 10. AVERAGE TOTAL NUMBER OF FILAMENTOUS PHYTOPLANKTERS AND AVERAGE NUMBER OF BLUE-GREEN ALGAE PER CUBIC METER ON EACH SAMPLING DAY, AND PHOTOSYNTHETIC QUOTIENT (Q_p) IN POND 3, 1968.

Month	Total Number	Blue-Green	% Blue-Green	Q_p
J	62,129	56,426	90.8	1.2
F	39,588	36,179	91.4	1.5
M	85,426	45,526	53.3	1.9
A	328,338	155,582	47.4	2.0
M				2.2
J	*Lyngbya* too numerous and matted to count			2.3
J				2.5
A	48,341	47,315	97.9	2.6
S	54,379	52,830	97.2	2.1
O	42,075	39,865	94.7	1.9
N	28,425	27,645	97.1	1.9
D	8,525	8,005	93.9	0.4

TABLE 11. AVERAGE TOTAL NUMBER OF FILAMENTOUS PHYTOPLANKTERS AND AVERAGE NUMBER OF BLUE-GREEN ALGAE PER CUBIC METER ON EACH SAMPLING DAY, AND PHOTOSYNTHETIC QUOTIENT (Q_p) IN POND 4, 1968.

Month	Total Number	Blue-Green	% Blue-Green	Q_p
J	46,700	13,461	28.8	0.9
F	65,193	18,965	29.1	1.2
M	103,387	21,427	20.7	1.7
A	645,045	232,392	36.0	1.7
M	941,413	457,527	48.6	1.6
J	301,210	301,210	100.0	2.7
J	259,731	256,463	98.7	2.7
A	25,374	25,374	100.0	1.9
S	4,592	4,592	100.0	1.0
O	5,883	5,883	100.0	0.9
N	1,907	1,863	97.7	0.9
D	1,745	1,745	100.0	0.6

occurs, can be as high as 3.3. Rabinowitch and Govindjee (1969) and Fogg (1968) pointed out that as cells mature, the photosynthetic product changes from protein and carbohydrate to highly reduced substances, such as fats and "lipidlike" substances. Bassham and Jensen (1967) stated that as much as 30 per cent of the CO_2 taken up by algae could be incorporated into fats.

Fat production by mature cells is stimulated by light. Spoehr and Milner (1949) demonstrated that *Chlorella pyrenoidosa* contained greatest fat stores when grown under high light intensity, and Lund (1969) suggested that, unlike other types of algae, blue-green algae thrive under intense illumination and high temperatures. Walsh (1971) showed that the amount of short-wave radiation that entered the ponds was greatest during the blue-green algae blooms.

Fat contains more energy than protein or carbohydrate. According to Morowitz (1968) their Gibbs free energy values are; protein: 5.50 kcal/g, carbohydrate: 4.10 kcal/g, and fat: 9.30 kcal/g. Increased photosynthetic quotients and quantities of light energy impinging upon the ponds in summer suggest that fat production also increased at that time. Increased production of fat may explain how the energy stored during CO_2 incorporation increased in the summer, and suggests a mechanism whereby relatively large amounts of energy can be incorporated into eutrophic systems.

SUMMARY

The amounts of solar energy absorbed by photosynthetic processes in four small ponds in northwestern Florida in 1968, estimated from diel and annual oxygen data, were compared with energy required for fixation of CO_2. On an annual basis, energy required for oxygen evolution and for fixation of CO_2 varied widely between ponds and within any one of them. Efficiencies of energy incorporation also varied greatly. For example, the mean annual efficiencies of absorption of light, calculated from oxygen data, were between 0.7 and 3.1% of incident solar radiation. Average annual efficiencies of absorbed solar energy incorporation into organic molecules, calculated from CO_2 data were between 3.2 and 26.8%.

Blue-green algae were the dominant phytoplankters in all ponds during most of the year and attained greatest numbers between April and October. Photosynthetic quotients were highest during the blue-green algae blooms. It is hypothesized that increased production of fat may explain why the energy required for CO_2 fixation increased during the summer.

REFERENCES

Arnon, D. I., H. Y. Tsujimoto, B. H. McSwain, and R. K. Chain. 1968. Separation of Two Photochemical Systems of Photosynthesis by Fractionation of Chloroplasts. In: Comparative Biochemistry and Biophysics of Photosynthesis (K. Shibata, A. Takamiya, T. A. Jagendorf, and R. C. Fuller, eds.). University Park Press, College Station, Pennsylvania. p. 113-132.

Bassham, J. A. 1965. Photosynthesis: The Path of Carbon. In: Plant Biochemistry (J. Bonner and J. E. Varner, eds.). Academic Press, New York. p. 875-902.

Bassham, J. A. and R. G. Jensen. 1967. Photosynthesis of Carbon Compounds. In: Harvesting the Sun: Photosynthesis in Plant Life (A. San Pietro, F. A. Greer, and T. J. Army, eds.). Academic Press, New York. p. 79-110.

Clayton, R. K. 1965. Molecular Physics in Photosynthesis. Blaisdell Pub. Co., New York. 205 p.

Fogg, G. E. 1968. Photosynthesis. American Elsevier Publishing Company, Inc., New York. 116 p.

Goldman, C. R., D. T. Mason, and B. J. B. Wood. 1963. Light Injury and Inhibition in Antarctic Freshwater Phytoplankton. Limnol. Oceanogr. 8:313-322.

Hartman, R. T. and D. L. Brown. 1967. Changes in Internal Atmosphere of Submersed Vascular Hydrophytes in Relation to Photosynthesis. Ecology 48:252-258.

Kahn, L. and F. T. Brezenski. 1967. Determination of Nitrate in Estuarine Waters: Comparison of a Hydrazine Reduction with a Brucine Procedure and Modification of a Brucine Procedure. Environ. Sci. Technol. 1:488-491.

Kalff, J. 1969. A Diel Periodicity in the Optimum Light Intensity for Maximum Photosynthesis in Natural Phytoplankton Populations. J. Fish. Res. Bd. Canada 26:463-468.

Kok, B. 1965. Photosynthesis: The Path of Energy. In: Plant Biochemistry (J. Bonner and J. E. Varner, eds.). Academic Press, New York. p. 903-960.

Lee, G. F. and A. W. Hoadley. 1967. Biological Activity in Relation to the Chemical Equilibrium Composition of Natural Waters. In: Equilibrium Concepts in Natural Water Systems (R. F. Gould, ed.). American Chemical Society, Washington, D. C. p. 319-338.

Lund, J. W. G. 1969. Phytoplankton. In: Eutrophication: Causes, Consequences, Correctives. U. S. Nat. Acad. Sci., Washington, D. C.

McAllister, C. D. 1961. Observations on the Variation of Planktonic Photosynthesis With Light Intensity, Using Both O_2 and C^{14} Methods. Limnol. Oceanogr. 6:483-484.

Morowitz, H. J. 1968. Energy Flow in Biology. Academic Press, New York. 179 p.

Rabinowitch, E. and Govindjee. 1969. Photosynthesis. John Wiley and Sons, Inc., New York. 273 p.

Rainwater, F. H. and L. L. Thatcher. 1960. Methods for Collection and Analysis of Water Samples. Geological Survey Water-Supply Paper 1454. U. S. Government Printing Office, Washington, D. C. 301 p.

Ryther, J. H. 1956. Photosynthesis in the Ocean as a Function of Light Intensity. Limnol. Oceanogr. 1:61-70.

Schindler, P. W. 1967. Heterogeneous Equilibria Involving Oxides, Hydroxides, Carbonates, and Hydroxide Carbonates. In: Equilibrium Concepts in Natural Water Systems (R. F. Gould, ed.). American Chemical Society, Washington, D. C. p. 196-221.

Spoehr, H. A. and H. W. Milner. 1949. The Chemical Composition of Chlorella: Effect of Environmental Conditions. Plant Physiol. 24:120-149.

Steemann Nielsen, E. and V. K. Hansen. 1961. Influence of Surface Illumination on Plankton Photosynthesis in Danish Waters (56° N) Throughout the Year. Physiol. Plant. 14:595-613.

Strickland, J. D. H. 1965. Production of Organic Matter in the Primary Stages of the Marine Food Chain. In: Chemical Oceanography, Vol. 1 (J. P. Riley and G. Skirrow, eds.). Academic Press, New York. p. 478-610.

Strickland, J. D. H. and T. R. Parsons. 1965. A Manual of Sea Water Analysis, 2nd Ed. Fish. Res. Board Canada Bull. No. 125. 203 p.

Verduin, J. 1956. Energy Fixation and Utilization by Natural Communities in Western Lake Erie. Ecology 37:40-50.

Walsh, G. E. 1971. Energy Budgets in Four Ponds in Northwestern Florida. Ecology 52:298-304.

Walsh, G. E. and P. T. Heitmuller. 1971. Uptake and Effects of Dichlobenil in a Small Pond. Bull. Environ. Contam. Toxicol. 6:279-288.

Welch, H. E. 1968. Use of Modified Diurnal Curves for the Measurement of Metabolism in Standing Water. Limnol. Oceanogr. 13: 679-687.

Wright, J. C. and I. K. Mills. 1967. Productivity Studies on the Madison River, Yellowstone National Park. Limnol. Oceanogr. 12:568-577.

HETEROINHIBITION AS A FACTOR IN *ANABAENA FLOS-AQUAE* WATERBLOOM PRODUCTION

Llewellyn R. Williams

INTRODUCTION

Late-summer, warm water conditions often give rise to waterblooms of blue-green algae in New Jersey ponds and lakes. *Anabaena flos-aquae* is frequently the major constituent of these blooms, often appearing, at least briefly, in codominance with *Microcystis aeruginosa*. That such blooms are an annual occurrence in some waters, while appearing only occasionally in similar waters nearby, suggests that we are dealing not with a single causative factor, but rather with an interaction of physical and chemical conditions leading to bloom production. Attempts to generalize the waterbloom phenomenon as a single cause-response relationship have been unsuccessful.

I would like to suggest the following mechanism for the production of waterblooms of *Anabaena flos-aquae*, based upon my studies in the laboratory, as well as natural system studies carried out in Lake Nelson:

1. Trigger for initial growth is the response of *Anabaena* to the advance of warm surface waters deeper into the water column and/or the direct benthic warming from penetrating solar radiation. A filament grows from the akinete, or overwintering cell of *Anabaena*, lying on the bottom; the early filament competes successfully in late-summer waters.
2. *A. flos-aquae* secretes into the water a heteroinhibitory compound; this active substance inhibits the growth of a broad spectrum of algal species in the community.

3. Upon death and lysis of the inhibited cells, inorganic and organic compounds are liberated into the system, providing stimulation to the advancing *Anabaena* population through nutrient or growth-factor effects.
4. The resultant high concentration of *Anabaena* at the surface, largely the result of extensive gas-filled pseudovacuole formation, is recognized as a waterbloom; it is composed of senescent *Anabaena flos-aquae* filaments.

MATERIALS AND METHODS

Laboratory Studies

Anabaena flos-aquae, obtained as axenic culture No. 1444 (U. of Indiana), was cultured in liter batches under constant light of 200-300 ft. candles and constant agitation with magnetic stirring. A mineral medium high in available nitrogen, phosphorus and sodium was used throughout:

Chemical Compound	Concentration (mg/l)
$NaNO_3$	2000
$MgSO_4 \cdot 7H_2O$	25
K_2HPO_4	250
NH_4Cl	50
$CaCl_2 \cdot 2H_2O$	50

The one-liter cultures appeared to peak in density at about 21 days and remained stable for several weeks before degenerating. When bubbled air agitation was substituted for magnetic stirring, early population declines were noted. Standard aseptic techniques were used throughout the study; cultures which failed to produce contaminant growth on glucose-supplemented or Bacto-Agar media were considered axenic.

Mineral medium in which *Anabaena* has been grown and removed by glass-fiber filtration is the basis for test growth media. These cell-free "conditioned" media were derived from cultures in log growth phase, usually 7-10 days after inoculation. No microscopic evidence of cell lysis was noted at this stage. Table 1 presents the test media and algae used in the various laboratory studies, noting any treatments or extraction procedures modifying the basic "conditioned" medium.

A simple $CHCl_3$-MeOH extraction procedure was used to remove lipid elements from conditioned medium for subsequent GLC analysis. The remaining lipid-free medium, cleared of residual solvent by flash evaporation, is called "extracted conditioned" medium; it is subsequently handled the same as the conditioned medium against which it is compared.

Scenedesmus dimorpha was the primary test organism used to evaluate the toxicity of Anabaena excretion products. *Scenedesmus* spp. are strong summer dominants in my study pond. Other test algae representative of pre-bloom genera included *Pandorina morum*, *Euglena gracilis*, *Platydorina caudata* and a *Chlorococcum* sp.

Inhibition tests were run in rotating culture apparatus which I designed for this purpose (Figure 1). The rotating turntable holds sixteen 1" diameter spectrophotometer tubes (Bausch and Lomb) which serve as culture tubes. The position and rotation of the tubes assure continuous and uniform illumination of approximately 300 ft. candles, depending upon the age of the circular fluorescent tube. To each tube is added 15 ml of test culture medium, a small magnetic stir-bar, and a cotton plug. The magnetic stir-bars provide periodic agitation in each culture tube as it passes over a magnetic stirring unit. The culture tubes with medium, stir-bar, and cottonplug in place are autoclaved as a unit prior to inoculation with test algae.

Table 1. CONDITIONS OF LABORATORY INHIBITION STUDIES

Study	Test Organism	Test Growth Medium
1	S. dimorpha	(a) $CHCl_3$-MeOH extracted conditioned (b) Conditioned
2	S. dimorpha	(a) P.M.M. (b) P.M.M. with $CHCl_3$-MeOH extract added
3	S. dimorpha	(a) $CHCl_3$-MeOH extracted conditioned (b) as (a) but with extract returned
4	S. dimorpha	(a) $CHCl_3$-MeOH extracted conditioned (b) MeOH treated conditioned (c) conditioned
5	S. dimorpha	(a) $CHCl_3$-MeOH extracted conditioned (b) Conditioned (Autoclaved) (c) Conditioned (Sterile-filered)
6	S. dimorpha	(a) P.M.M. (b) P.M.M. plus Oleic acid (c) P.M.M. plus Linoleic acid (d) P.M.M. plus Linolenic acid
7	S. dimorpha	(a) $CHCl_3$ extracted conditioned (b) Diethyl ether extracted conditioned (c) Hexane extracted conditioned (d) Conditioned
8	S. dimorpha	(a) Hexane extracted conditioned (b) Hexane treated conditioned
9a	E. gracilis	(a) $CHCl_3$-MeOH extracted conditioned (b) Conditioned
9b	Chlorococcum sp.	
9c	P. morum	
9d	P. caudata	
10	Lake Nelson phytoplankton	(a) $CHCl_3$-MeOH extracted conditioned plus early-bloom extract (b) $CHCl_3$-MeOH extracted conditioned plus late-bloom extract
11	Lake Nelson phytoplankton	(a) $CHCl_3$-MeOH extracted conditioned (b) as (a) but with extract returned
12	S. dimorpha A. Flos-aquae	(a) Early-bloom lake water (Mineral supplemented) (b) Late-bloom lake water (Mineral supplemented)

Note: P.M.M. = Pure mineral medium

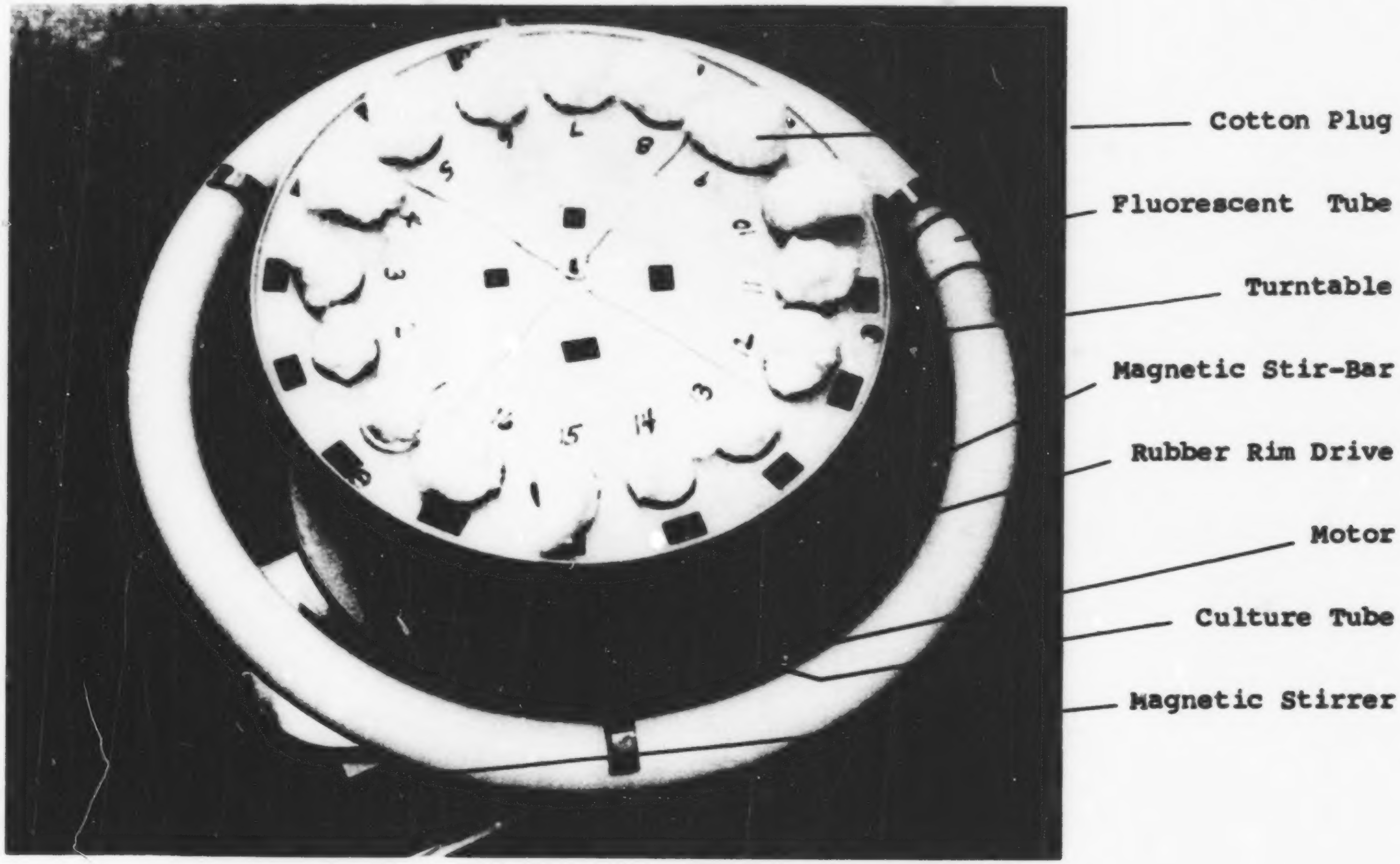

Cotton Plug
Fluorescent Tube
Turntable
Magnetic Stir-Bar
Rubber Rim Drive
Motor
Culture Tube
Magnetic Stirrer

Figure 1. Rotating culture apparatus

The length of an experimental run varied from three days to as long as a week. At the termination of each growth study all culture tubes were vigorously agitated. A 1 ml aliquot was removed from each, diluted to 10 ml with distilled water and run through a 24 mm diameter Millipore filter (0.45u, HAWP) and dried. The filters were cleared with cedar oil and the test organisms enumerated by the method of McNabb (1960), utilizing an ocular grid as a counting aid against the plain Millipore filters.

Natural System Studies

Lake Nelson, in Middlesex Co., New Jersey, which regularly exhibits waterblooms of blue-green algae, was selected for my field study. This eutrophic impoundment of about 15 acres surface area has a maximum depth of about 2 meters.

Phytoplankton and zooplankton composition were monitored on a daily basis through two consecutive waterblooms. Air and water temperatures were similarly monitored and a weather log was maintained for this period. Total plankton samples were preserved with acid Lugol's solution and enumerated at 100X magnification in a counting chamber. Net plankton samples were usually counted fresh. Plankton and filtered water samples were extracted with $CHCl_3$-MeOH for fatty acid analysis. Gas chromatographic analysis of extracts was performed on a Micro-Tek MT-220 unit equipped with flame ionization detectors, polar and non-polar columns. Quantitative estimates were obtained with a Disc Integrator.

RESULTS

Laboratory Studies

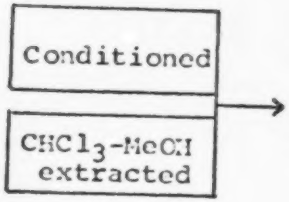
An initial comparison between Scenedesmus growth in conditioned medium and $CHCl_3$MeOH extracted conditioned medium showed highly significant differences in growth between the two media; less growth is noted in the conditioned medium (Table 2a). In an attempt to demonstrate inhibitory

TABLE 2. SUMMARY OF LABORATORY RESULTS

RATIONALE

Is the inhibitor lipid in nature? Tested by lipid extraction of conditioned medium, comparision with test organism growth in unextracted conditioned medium.

2a.


Conditioned
$CHCl_3$-MeOH extracted

TEST RESULTS

(1) Conditioned medium inhibitory.
(2) Lipid extraction removes inhibition.
(3) Excludes mineral nutrient inequity effects. $P > .99$

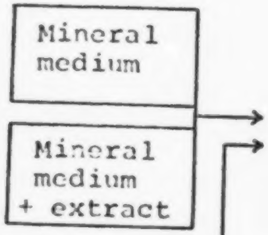
Lipid extraction removed inhibition.

Tested extract *per se* for activity.

(1) Added to pure mineral medium

(2) Returned to extracted conditioned medium.

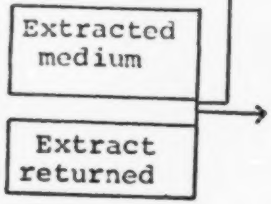
2b.


Mineral medium
Mineral medium + extract

Extract does *not* retain potency.

No difference; suggests that inhibitor is either unstable lipid or nonlipid.

2c.


Extracted medium
Extract returned

No difference

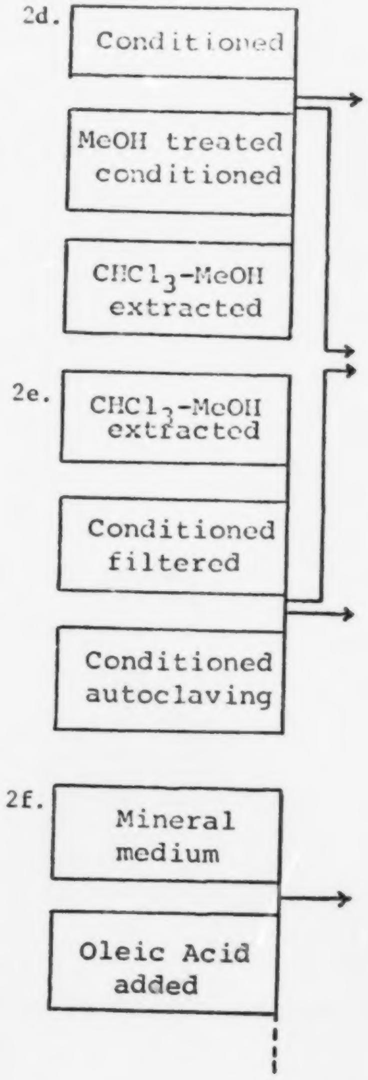
TABLE 2 (CONTINUED). SUMMARY OF LABORATORY RESULTS

If not lipid, is it proteinaceous?

Tested by:

(1) Methanolic treatment.

(2) Heat treatment (autoclaving) of conditioned medium.


2d.
Conditioned
MeOH treated conditioned
$CHCl_3$-MeOH extracted
2e.
$CHCl_3$-MeOH extracted
Conditioned filtered
Conditioned autoclaving
2f.
Mineral medium
Oleic Acid added

Both conditioned media equally inhibitory.

Tested against extracted: $P > .95$

Protein ruled out as inhibitor.

Both conditioned media equally inhibitory.

Tested against extracted: $P > .99$

Is the inhibitor an unsaturated fatty acid unstable to extraction and/or subsequent handling?

No difference.

No activity against test alga by any fatty acids tested.

TABLE 2 (CONTINUED). SUMMARY OF LABORATORY RESULTS

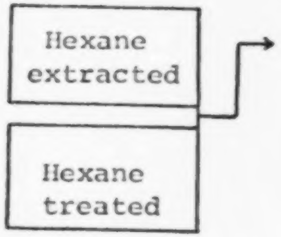
Tested by addition of dominant unsaturated fatty acids at levels occurring in Anabaena culture.	2f. Linoleic Acid added Linolenic Acid added	→ No difference	No activity against test alga by any fatty acids tested.
If the solvent extraction is inactivating the inhibitor, would other organic solvents do the same? Possible key to nature of the compound, assess growth in conditioned media extracted with solvents of different polarity.	2g. $CHCl_3$ extracted Ether extracted Hexane extracted Conditioned	→ Growth in: $CHCl_3 \rangle$ Ether $\rangle$ Hexane $\rangle$ Conditioned $P > .95$ $P > .95$ $P > .95$ Increased polarity increases removal of inhibition.	

TABLE 2 (CONTINUED). SUMMARY OF LABORATORY RESULTS

If extraction per se inactivates inhibitor, will "treatment" with organic solvent prove as effective as physical separation of the phases?

Tested by comparing hexane extraction with hexane treatment.

2h.

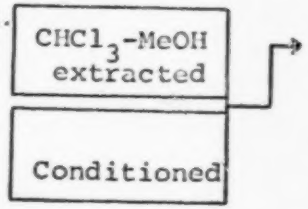

Hexane extracted
Hexane treated

No difference.
Consistent with inactivation at the water:organic solvent interface.

What is the response of other test algae to conditioned medium?

Tested Euglena, Chlorococcum, Pandorina and Platydorina for growth in conditioned and extracted conditioned media.

2i.


CHCl3-MeOH extracted
Conditioned

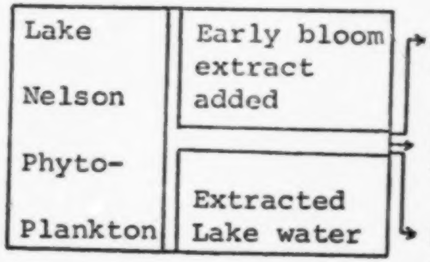
All inhibited by conditioned medium.
Morphological and cytological differences noted.

(Figure 2; Plate 1-4)

Do organic compounds in natural waters during Anabaena water blooms affect phytoplankton growth?

Growth in the presence and absence of early bloom extract is compared.

2j.


Lake Nelson Phyto- Plankton
Early bloom extract added
Extracted Lake water

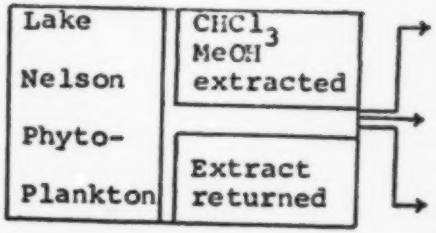
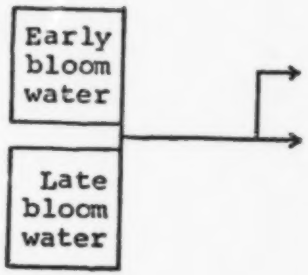
Anabaena the only alga stimulated by early bloom extract.

Anacystis, Pandorina favored without extract.

No difference for seven other genera.

(Figure 3)

TABLE 2 (CONTINUED)- SUMMARY OF LABORATORY RESULTS

How do the same phytoplankters respond to lab Anabaena extract? Tested by returning extract to extracted medium, assaying activity.	2k.  Lake Nelson Phyto-Plankton $CHCl_3$ MeOH extracted Extract returned	Anabaena not stimulated by its own extract. Micractinium better without extract. Pandorina, Eudorina, Scenedesmus and Chlorogonium better with extract. (Same do well at end of water bloom) (Figure 4)
What is effect of filtered lake water from a waterbloom on the growth of Anabaena and Scenedesmus? Tested by co-growing in early bloom and late bloom water (both mineral supplemented).	21.  Early bloom water Late bloom water	Growth in: Early bloom > for Anabaena Late bloom > for Scenedesmus Anabaena growth better in early than late bloom water, while Scenedesmus is favored in late bloom water, consistent with natural system findings. (Figure 5)

activity believed to be contained in $CHCl_3$-MeOH extract from the conditioned medium, I added the extract to pure mineral medium, comparing Scenedesmus growth to mineral medium without extract supplement (Table 2b). No difference was noted in the growth in these two media. Upon returning $CHCl_3$-MeOH extract to the conditioned medium from which it had been removed, no difference in growth of Scenedesmus could be attributed to extract activity when compared with growth in extracted conditioned medium (Table 2c). Removal of extract removed activity, but return did not restore the inhibitory properties to either medium.

To screen proteinaceous materials from consideration as the source of activity in the conditioned medium, I compared conditioned medium which was autoclaved, with that which was sterile-filtered; autoclaving had no effect upon the activity present in the conditioned medium (Table 2e). Both conditioned media showed significant reduction in growth when compared to the extracted control. In subsequent studies activity was retained with up to 60 minutes of autoclaving, the longest period evaluated. Methanol treatment had no effect upon activity (Table 2d). Linoleic, oleic, and linolenic acids were tested for inhibition of Scenedesmus growth at levels corresponding to their occurrence in conditioned medium. No activity was noted (Table 2f).

The action of three different solvent extractions in effecting removal of activity from conditioned medium are compared in Table 2g. All solvents tested remove sufficient activity to allow growth significantly higher than conditioned medium. The activity is removed more completely in proceeding from less polar to more polar solvent extraction. While substantial reduction in activity is provided by ether or chloroform extractions, inhibition appears to be more completely removed with the highly polar $CHCl_3$-MeOH solvent system noted earlier.

Table 2h shows results of a test comparing the effect of hexane extraction with hexane treatment--hexane is added, then removed by evaporation. No difference was noted between the abilities of these media to support the growth of *Scenedesmus*, even though hexane extraction had been shown to remove activity.

Representatives of 4 genera found in Lake Nelson in July-August 1970, were substituted for *Scenedesmus* to serve as indicators of the inhibitory activity of the conditioned medium. *Pandorina* and *Platydorina* were completely eliminated in tubes containing conditioned medium. *Euglena* and *Chlorococcum* were present in reduced numbers, but showed evidence of cytological and morphological changes in conditioned medium relative to the extracted controls. A loss of flagella, vacuolate appearance and spherical shape were noted in *Euglena* grown in conditioned medium. The absence of actively dividing cells was seen in *Chlorococcum* grown in conditioned medium. In addition, individual cells showed increased density consistent with continued "assimilation without division." (See Figure 2)

Two parallel laboratory studies were performed in an effort to determine the response of natural Lake Nelson phytoplankton to $CHCl_3$-MeOH extracts of (1) early-bloom lake water, and (2) laboratory conditioned medium. In comparing phytoplankton growth in the presence or absence of early bloom lake water extract (Figure 3), note that only *Anabaena* grew better with addition of the extract than without. The difference was highly significant ($P>.99$). No difference in growth was noted for seven other genera. However, *Anacystis* and *Pandorina* growth was greater without the lake water extract.

The extract from laboratory conditioned medium did not stimulate *Anabaena* growth, *Micractinium* grew better without the added extract; *Pandorina*, *Eudorina*, *Scenedesmus* and *Chlorogonium* grew better in the presence of extract (Figure 4). Interestingly, in the natural system, the last

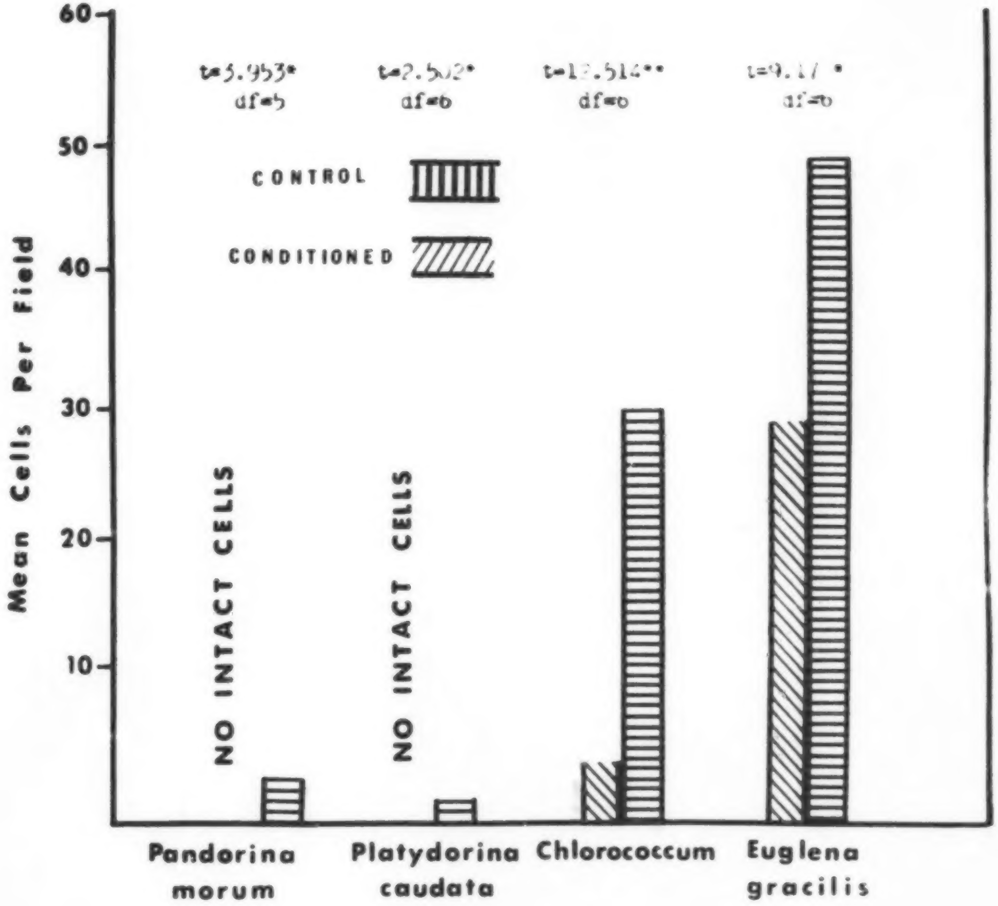


Figure 2. Inhibition Study 9--Comparative growth of four representative genera of algae in conditioned medium and extracted conditioned (control) medium. Values represent the average of mean cell counts per field for 4 tubes in each treatment combination.

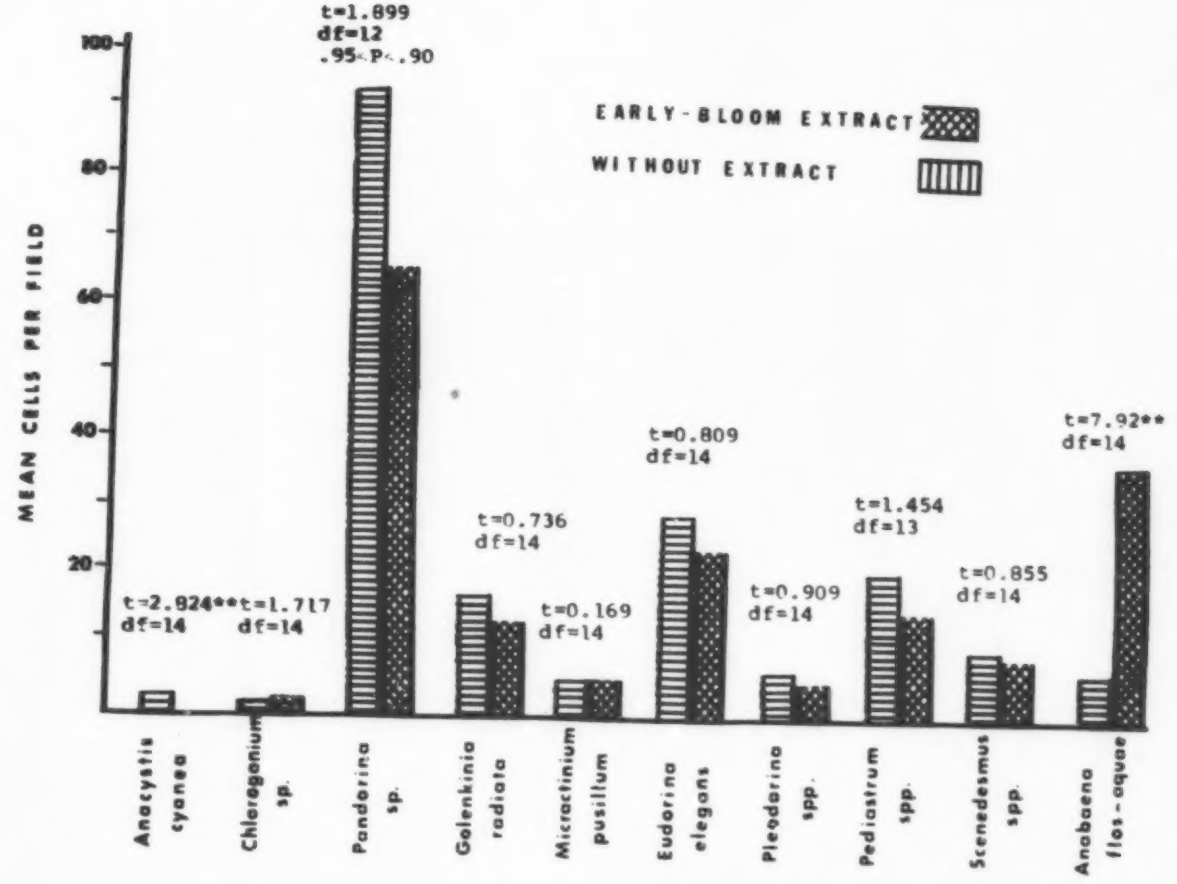


Figure 3. Study 10--Comparison of growth of natural Lake Nelson phytoplankton in extracted pond water with and without early bloom lipid extract. Values are 8-tube averages of mean cell counts per field.

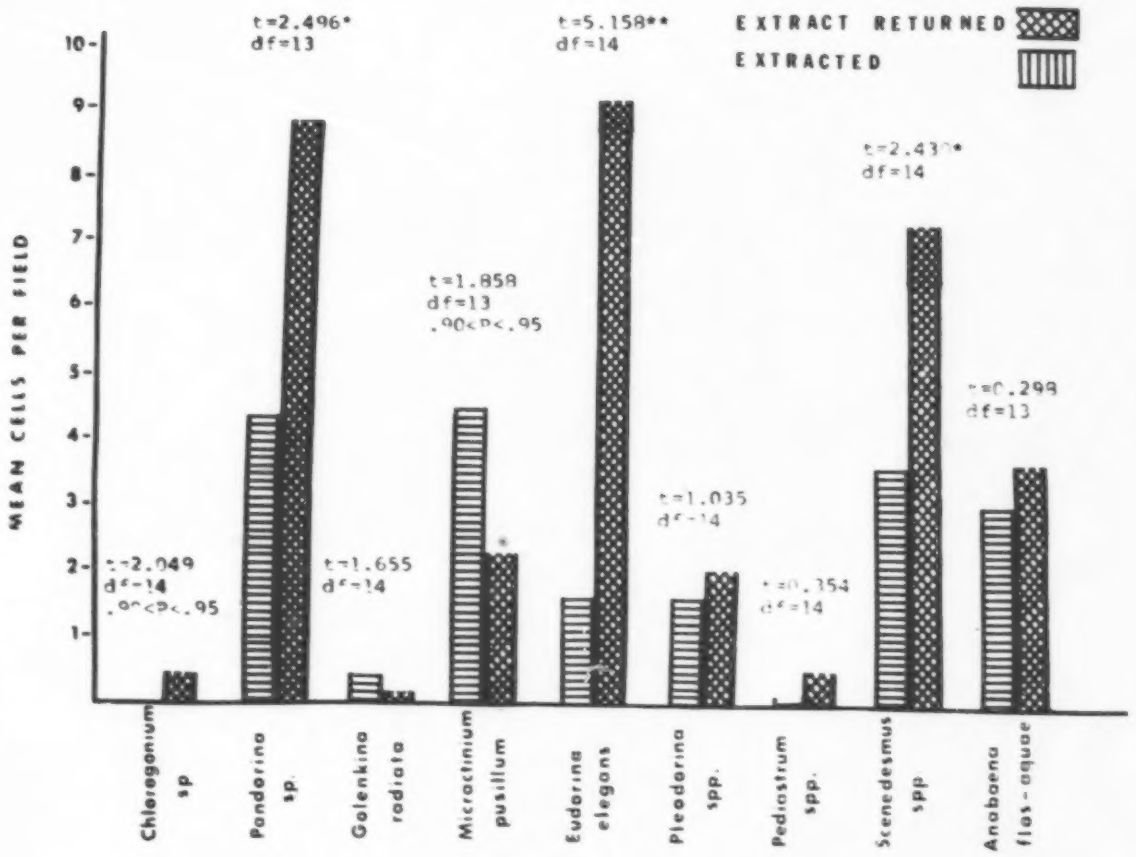


Figure 4. Study 11--Comparative growth of natural Lake Nelson phytoplankton in extracted conditioned medium and extracted conditioned medium with extract returned. Values are 8-tube averages of mean cell counts per field.

four algae showed moderate to strong growth responses during decline of the second Anabaena waterbloom in 1970 suggesting stimulation by lipid materials released from Anabaena.

To determine whether dissolved organics in the natural lake water would mediate competition between major algal constituents, I ran co-growth studies with Anabaena and Scendesmus using waters from early-bloom and late-bloom lake conditions. The results of these experiments, followed on a daily basis, are shown in Figure 5. Note that Scenedesmus grown in early-bloom water varies little from the initial inoculum concentration, while the Anabaena is "favored" under these conditions. Mineral supplements to the lake waters minimized any role of inorganic ion depletion in mediating the differential response noted.

Natural System Observations

The growth and early decline of constituent phytoplankton populations is illustrated in Figure 6 which depicts the mean cell counts of all other species of algae contrasted with the growth curve of Anabaena flos-aquae in Lake Nelson. Response of zooplankton during the bloom is shown in Figure 7. Percent composition of all identified plankton populations, derived by direct cell counts of daily net plankton samples, is included in the Appendix. Comparison of individual phytoplankton population responses to the growth of Anabaena is also included in the Appendix.

Two major weather disturbances - heavy, prolonged thunderstorms - significantly altered the phytoplankton succession pattern during the sampling period. The first of these, on August 1, essentially removed what remained of the first Anabaena bloom. Wind effects can be noted in the daily counts; precipitous drops in Anabaena concentration followed periods during which strong west wind activity piled surface algae at the opposite

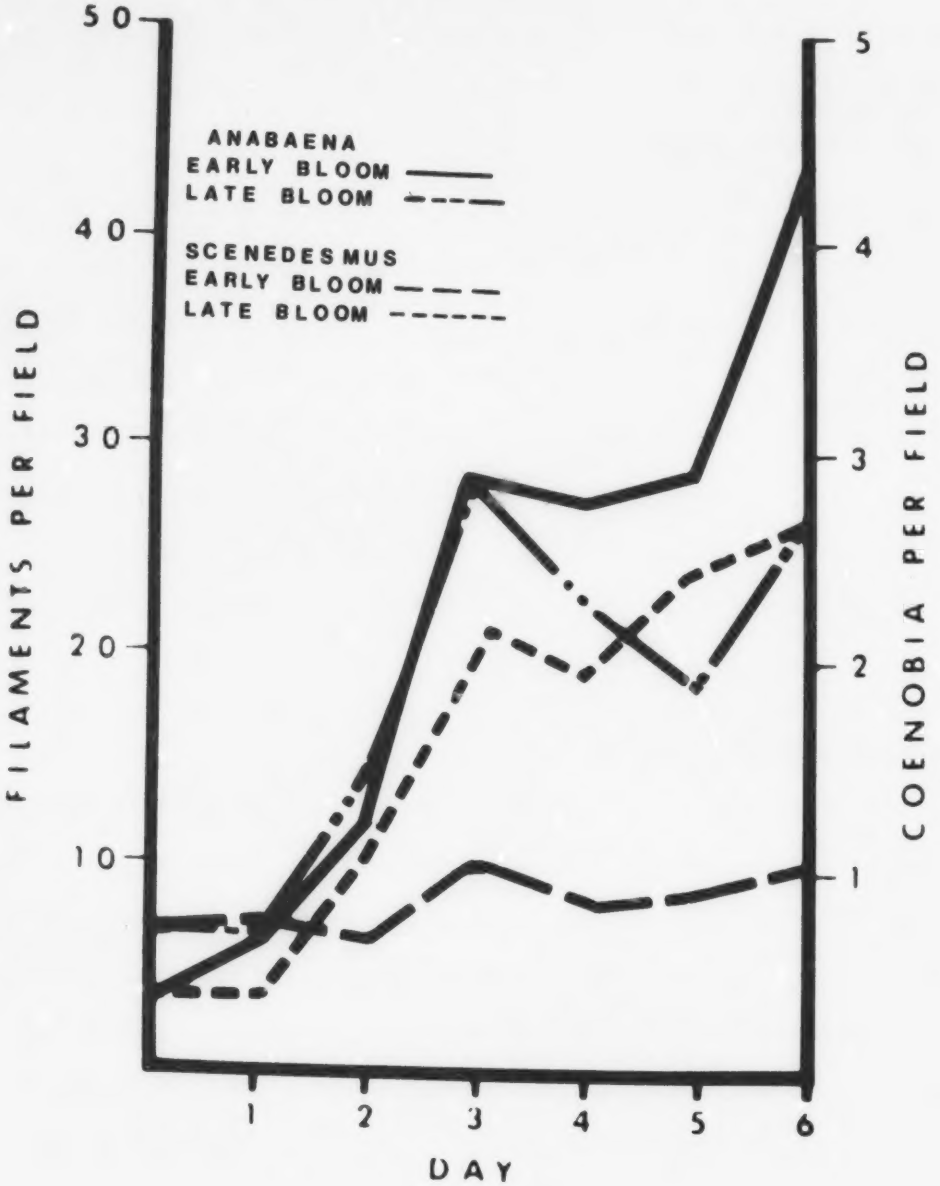


Figure 5. Study 12--Comparison of Anabaena flos-aquae and Scenedesmus dimorpha grown together in early-bloom (8 Aug.) and late-bloom (25 Aug.) filtered pond water (Lake Nelson, 1970). Points represent 8-tube averages of mean coenobium or filament counts per field. Counts of membrane filter-trapped algal samples were made daily.

	Day 1	Day 3	Day 6
Scenedesmus	t=0.278 df=14	t=4.628* df=14	t=4.588* df=13
Anabaena	t=1.248 df=14	t-1.136 df=13	t=6.570* df=12

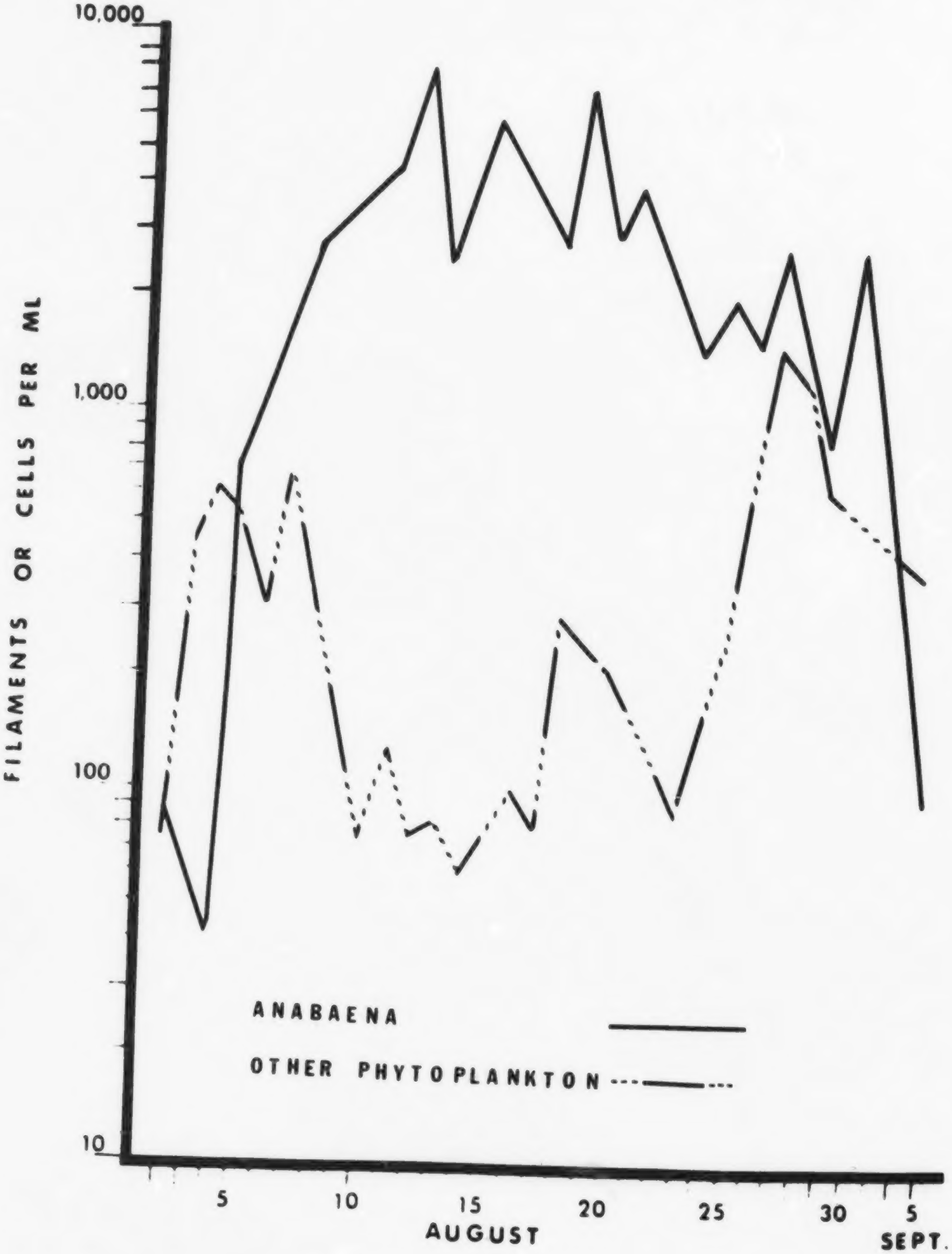


Figure 6. Mean variation of phytoplankton components during an *Anabaena flos-aquae* waterbloom in Lake Nelson, 1970.

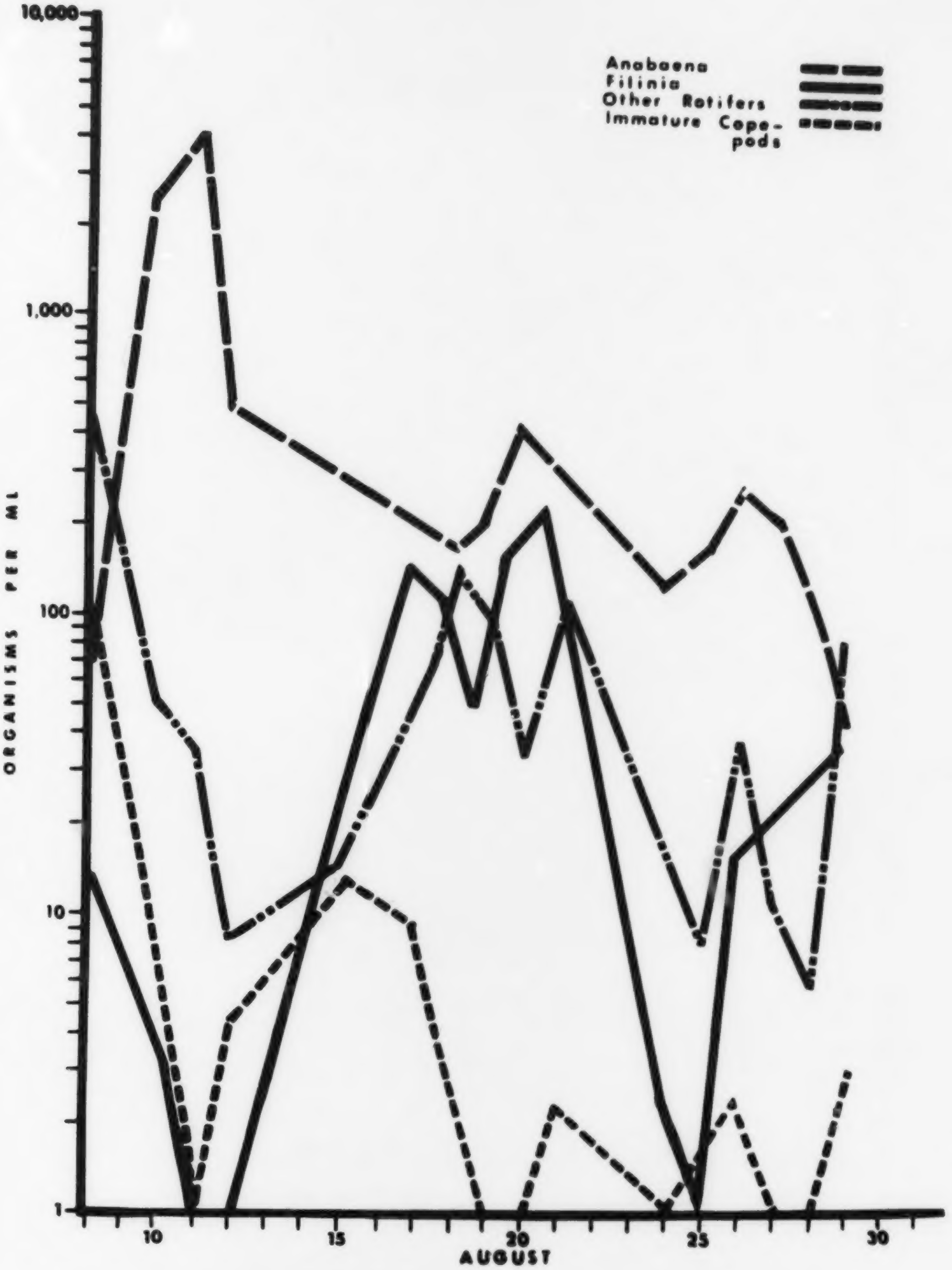


Figure 7. Variation of zooplankton components during an Anabaena flos-aquae waterbloom in Lake Nelson. Counts were made from plankton net concentrates (1970).

end of the pond. High concentrations noted on July 19 followed several days of easterly breezes piling algae against the dam at my sampling site. Heavy concentrations spilling over the dam resulted in a fish-kill in the receiving brook July 21.

Attempts to culture *Anabaena* or *Microcystis* from surface waters during waterbloom formation were singularly unsuccessful. Growth was obtained only with *Anabaena* from the shallow waters of the inlet brook to Lake Nelson. In sampling these waters the bottom sediments were disturbed and silt entered the samples.

Gas Chromatography

I analyzed the free fatty acids of daily cell extractions by gas chromatography through the development of an *Anabaena* waterbloom. No attempt was made to measure the actual quantity of fatty acids per unit of raw sample; amounts are expressed as relative percentages of the fatty acids analyzed. GLC analyses of extracts from filtered lake water are included where available (Table 3). Odd-chain fatty acids are plotted separately in Figure 8.

DISCUSSION

Let us examine the evidence in support of each step in the proposed mechanism for *Anabaena flos-aquae* water-bloom formation.

> Initiation of filament growth from akinetes deep in the water column is triggered by the advance of warm surface waters and/or the direct benthic warming from penetrating solar radiation. The early filament competes successfully in mid to late summer waters.

Healthy laboratory cultures of *Anabaena* regularly settled to the bottom of the flask upon removal of agitation. Cultures which have degenerated show some tendency to surface; this is more apparent with

TABLE 3

% COMPOSITION OF FATTY ACIDS FROM LABORATORY AND LAKE SOURCES

DATE	CARBON NUMBER						
	C-12	C-13	C-14	C-15	C-16	C-17	C-18
				LAKE NELSON, 1970			
7/29	11.5 (8.0)	18.0 (5.3)	11.4 (11.3)	9.8 (7.5)	18.2 (54.7)	21.4 (10.8)	5.1 (3.0)
7/30	9.3	4.6	7.2	2.0	33.4	5.1	30.9
8/1	15.8 (1.8)	5.3 (1.8)	4.2 (4.2)	0.5 (4.2)	10.5 (28.0)	Tr (22.9)	63.2 (36.4)
8/2	11.2 (3.7)	9.1 (9.1)	9.7 (4.3)	1.5 (5.5)	39.6 (18.8)	1.5 (14.4)	27.2 (43.2)
8/3	17.4	1.6	1.6	0.8	30.2	0.8	54.9
8/4	3.4	0.8	1.9	1.1	47.0	1.1	43.3
8/5	2.2 (4.8)	0.7 (20.5)	7.0 (16.9)	2.4 (Tr)	52.9 (22.9)	1.9 (8.2)	33.3 (26.7)
8/10	5.2 (11.0)	2.1 (9.0)	4.3 (9.5)	1.8 (1.2)	43.0 (31.6)	2.2 (10.6)	38.7 (27.0)
8/13	1.8 (5.4)	0.9 (5.3)	1.3 (5.5)	1.3 (9.2)	53.5 (32.0)	1.2 (18.9)	39.0 (23.8)
8/25	(11.4)	(15.3)	(9.3)	(1.2)	(20.3)	(7.6)	(17.4)
				BUDD LAKE, 1970			
7/29	(2.1)	(10.6)	(8.8)	(8.8)	(34.2)	(7.4)	(27.9)
				LABORATORY CULTURE OF A. FLOS-AQUAE			
	12.0 (7.0)	3.5 (1.8)	11.1 (6.1)	3.2 (0.5)	36.5 (16.5)	3.5 (3.3)	29.1 (65.7)

Values represent gas chromatographic analysis of 12 - 18-carbon fatty acid methyl esters from $CHCl_3$-MeOH extracts of cells (open) and cell-free (in brackets).

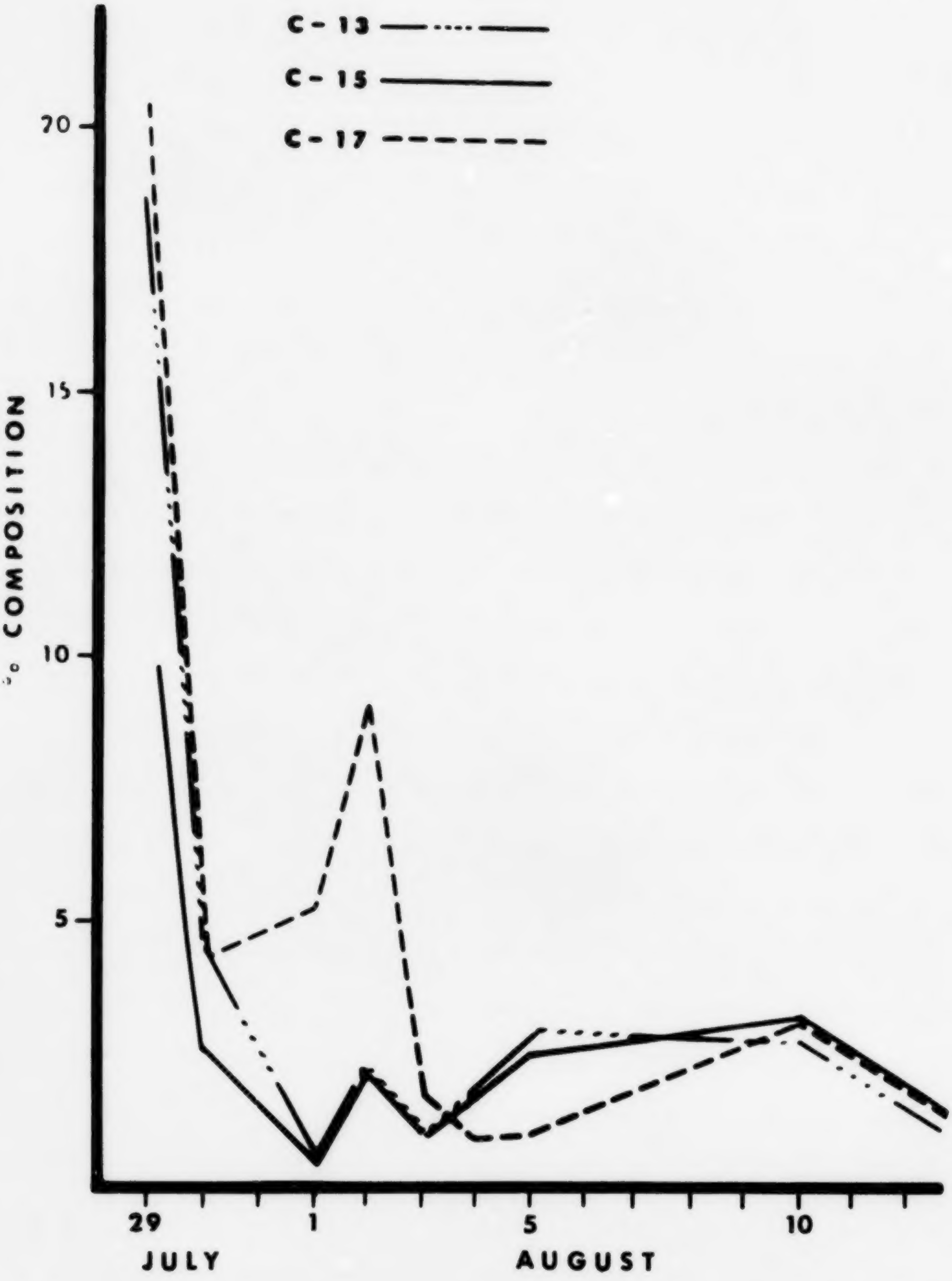


Figure 8. Variation in the percent composition of the total fatty acids analyzed represented by the odd-chain fatty acids C-13, C-15, and C-17. Values were obtained by gas chromatographic analyses of daily cell extracts taken through the onset of an Anabaena flos-aquae waterbloom.

pseudovacuole formation noted in natural populations. That surfacing filaments are not healthy, but rather degenerate and incapable of normal growth when placed in culture medium, is strongly suggested by culture studies of waterbloom samples taken from seven lakes and ponds in New Jersey from 1968 to 1970. The only filaments to grow successfully were obtained at the inlet to Lake Nelson; no surface film of *Anabaena* was present at this site. The sample contained some silt and benthic diatoms as well as viable *Anabaena* filaments.

I have found in my culture work that *Anabaena flos-aquae* is inhibited by light in excess of 400 foot-candles; under these conditions the filaments become chlorotic; rapid degeneration soon follows if the cultures are not removed to lower light conditions. Supraoptimal light and temperature in the surface waters may play a significant role in the senescence of cells in the surface film (Hutchinson, 1967).

In the Northeast blue-green blooms occur at a time when warm surface waters extend deep in the water column. In the summer of 1970 bloom reports began to come in from central and northern New Jersey weeks prior to their anticipated appearance. The surface water temperature of some of these lakes had reached 35°C after a sustained period of high insolation and high air temperatures. Optimum temperatures reported for the growth of *Anabaena* are, with few exceptions, far below the surface temperature noted for these lakes, but more closely approximate hypolimnetic temperatures. As warm summer waters approach benthic muds, large amounts of phosphate and sometimes nitrate and ammonia may be released into the water from the sediments (Hutchinson, 1967).

It is not at all unusual for blue-green algal waterblooms to be first "discovered" when surface filming has already begun. However, it is hardly possible to conceive of this sudden appearance of surface film based upon the reproductive rate for surface-dwelling filaments, subjected to unfavorable conditions of light and temperature. Accumulation at the surface, of filaments rising through the water column, can account for the rapidity of surface-film development as well as the "greening" of a lake noted prior to surface-film development in carefully watched waterblooms. If, as has been suggested, akinetes are the source of repopulation of Anabaena for the subsequent year, why can they not be found in surface waters prior to Anabaena waterbloom formation, if not for the fact that they are indeed resting on or in the sediments of the lake bottom.

The heterocyst-containing Anabaena filament has a competitive "edge" in late-summer waters through the mechanism of nitrogen-fixation. Fitzgerald(1970) found A. flos-aquae to fix N_2 actively when other sources of nitrogen were low. The waters classically hold relatively low nitrate concentrations during this period, while providing phosphate in adequate amounts to support phytoplankton growth. Dependence upon the oxidized forms of nitrogen may limit many phytoplankters under these conditions The autumnal pulse, provided by convection-borne nutrients when surface waters cool, is largely a result of nitrate replenishment in the upper waters. In studies of Anabaena cylindrica, Fogg (1949) found heterocyst production to be greater at low light intensities. He found also that some free sugars and organic acids stimulate the formation of heterocysts in this species. The presence of nitrate, ammonia or dissolved amino-acid-N tended to retard or inhibit heterocyst production.

Luxury uptake of phosphorus in blue-green algae is well-documented and a reproductive explosion can continue for many divisions after depletion of the phosphorus from surrounding waters. The dynamics of sediment-water phosphorus exchange would further tend to favor the growth of early Anabaena populations low in the water coulumn, or early filament production from akinetes on the sediment surface. Fitzgerald (1970) reports that the presence of iron can alter phosphorus solubilities shifting the equilibrium to favor algal growth at pH 9-9.5.

> *Anabaena* *flos-aquae* secretes into the water a heteroinhibitory compound; this active substance inhibits the growth of a broad spectrum of algal species in the community.

It was important initially, to establish that activity against other algal forms was a result of *Anabaena*-produced compounds excreted during active growth of the culture. Periodic spot-checks on bulk liquid cultures of the blue-green never revealed more than low-level background contamination when healthy growing cultures were examined under oil immersion. Upon degeneration of some cultures, I have noted strong increases in bacterial flora. Laboratory observations indicated that healthy *A. flos-aquae* cultures tend to keep bacterial contaminants at very low levels.

The conditioned medium, taken from the logarithmic growth phase with essentially no cell lysis, *is* biologically active, as the results of my inhibition studies consistently show, suggesting that release of active materials by cell breakdown is unnecessary. Evidence from co-growth experiments in early- and late-bloom water further suggests that much of the biological activity is *lost* late in the bloom when cell death and decomposition is high. Natural system studies in Lake Nelson show dramatic fall-off of several phytoplankton species prior to maximum concentration of *Anabaena* in the surface waters, but late-bloom recovery, in some instances, was just as dramatic, even though high numbers of *Anabaena* filaments remained. The long-term toxicity reported by Proctor (1957a) was not evident, although there was a shift noted in phytoplankton dominants, in comparing pre-bloom and post-bloom communities. Fogg (1952) found extracellular products of Anabaena to be liberated not by autolysis, but as an "invariable concomitant of growth". It was noted that Anabaena - conditioned medium was consistently inhibitory to all representative forms tested, relative to growth in extracted control medium. Microscopic examination of the algal forms suggested that division had been inhibited in Chlorococcum and Euglena gracilis.

In studies with natural phytoplankton, I found A. flos-aquae to be stimulated by the return of early-bloom lipid extract; no other algae were stimulated significantly.

The extract per se, when reintroduced to the extracted medium or to pure mineral medium, did not result in restoration of inhibitory activity. This has been a consistent finding, spurring attempts to explain the inactivation of the biologically active compound upon extraction. A comparison was made between hexane extraction, shown to remove a significant amount of biological activity and hexane "treatment", in which hexane was added, shaken up with the conditioned medium, and volatized rather than physically separated. That no difference was noted in the inhibitory properties of these media strongly suggests that the inactivation is accomplished at the interface of the aqueous-organic solvent compartments, rather than in subsequent handling of the extract. The strong possibility of an active chemical complex between hydrophyllic and lipophyllic moieties which separate into inactive substituents at the slovent-water interface was suggested by Litchfield (personal communication).

The inverse relationship of zooplankton response to Anabaena growth, suggested by my data, may reflect actual damage to the zooplankton population, or perhaps an avoidance behavior on the part of these motile forms to the products of surface concentrations of Anabaena. Such behavior has been suggested in studies by Hardy (1936) and Vance (1965).

If the inverse relationship of zooplankton to Anabaena should be a consistent finding, it could be recognized as a further competitive advantage in the ascent of Anabaena to dominance.

> Upon the death and lysis of inhibited cells, stimulation is provided to Anabaena flos-aquae by the nutrients and/or growth factors so liberated.

Co-growth studies showed enhanced growth of A. flos-aquae in lake water from early-bloom conditions, when compared with late-bloom water. The early-bloom water, obtained on August 8, represents a period of strong Anabaena ascendency, while most other species were declining. August 25, the date of the late-bloom water, represents a period of Anabaena decline, coupled with the recovery of other algal populations. The possibility of the relative successes of Anabaena in the co-growth studies being related to autoinhibition by self-produced compounds in the late-bloom waters was not overlooked. That Anabaena is stimulated by the presence of compounds in the lipid extract of early-bloom water was confirmed by comparing growth in extracted early-bloom lake water to that occurring in the same waters with the return of the lipid extract. In the absence of extractable lipid, Anabaena growth was modest at best. Self-produced lipid materials failed to stimulate Anabaena growth; the compound(s) stimulatory to Anabaena are produced from other sources.

Anabaena can use external organic compounds in synthesis, utilizing fructose and sucrose with high efficiency in formation of extracellular polysaccharides (Moore and Tischer, 1965). These authors demonstrated the presence of the enzymes, fructose diphosphate phosphatase, and fructose diphosphate aldolase. Anabaena readily photoassimilates acetate (Hoare, Hoare, and Moore, 1967), fatty acids--palmitic, stearic, and oleic--to be incorporated into lipids (Nichols, Harris, and James, 1965). Nichols, et al., found acetate to be largely incorporated into phosphatidyl glycerol, the major phospholipid of blue-green algae. In each of the studies mentioned uptake and destination of C^{14}-labeled sugars and organic acids were determined by radioassay of chromatographically separated products. Fogg (1952) found that Anabaena cylindrica continued to assimilate organic compounds for some time in the dark, finding also that organic acids and sugars stimulated N_2 fixation. Kuenzler (1970) found C^{14}-fructose to be taken up by blue-green algae at low CO_2 tension. Lange (1970) found a three-fold increase in Anabaena circinalis growth with the addition of sucrose to the mineral medium. These algal cultures were not axenic, and Lange suggests the role of bacteria in metabolizing the sucrose to CO_2, the latter representing the primary algal nutrient. He does not rule out direct utilization of sucrose by the Anabaena. Jakob (1961) found greatly increased growth of Nostoc in Chu medium with addition of glucose; "muscarine" activity persisted at a reduced level.

That Anabaena can utilize extracellular organic compounds is rather well-established; whether or not the algal community provides such a carbon source is another question. While it can be argued that there are sources for dissolved organic compounds in natural waters other than phytoplankton production, it is assumed by some researchers that the bulk of dissolved organics are phytoplankton-produced (Domogalla, et al., 1925; Hellebust, 1967). Fogg (1962) suggested that only a small percentage of these organics are liberated by active, growing cells, the major part arising from the decomposition of phytoplankton. Lefevre, et al., (1952) reports rapid degeneration of some algal species subjected to blue-green algae-produced inhibitors with cell lysis and consequent release of organic compounds. Fitzgerald (1970), working with *A. flos-aquae* (Ind. 1444), found the best source of nutrients in his study to be killed algal cells, live algae tending to hold onto their nutrients and providing less growth than the control mineral medium. Meeuse (1962) reports the reserve products of green algae to be true starches, sucrose and glucose. As most of the pre-bloom phytoplankton represents this group, sudden release of such reserves might certainly be expected to enhance *Anabaena* growth.

In view of the high efficiency with which some simple sugars and organic acids are reportedly assimilated, their role as potential carbon sources for *Anabaena* must not be underestimated, particularly under conditions of low CO_2 availability. That such conditions *do* occur in nature has been strongly stated by Kuentzel (1969). He has suggested that a mutualistic relationship exists between waterbloom-producing blue-green algae and bacteria, whereby the bacteria obtain oxygen and organic compounds (mucilages) in return for CO_2 utilized by the algae. Kuentzel further suggests that high BOD in natural waters may stimulate blue-green waterblooms by boosting the bacterial populations. Analyses of GLC peaks from cell extracts through the onset of an *Anabaena* bloom do not suggest high bacterial activity.

The presence of a high percentage of odd-chain fatty acids was noted in samples from the late degenerative phase of the first of two *Anabaena* waterblooms. Similar high odd-chain percentages were noted several weeks into a major *A. flos-aquae* bloom on Budd Lake. That such odd-chain fatty acids are largely bacterial in origin has been documented

(Parker, Van Baalen and Maurer, 1967). This suggests that the late-bloom degeneration is accompanied by high bacterial populations (not at all unexpected). However, if we extend the use of odd-chain fatty acids as a rough index of bacterial populations, we do not note evidence of bacterial blooms preceding the second Anabaena bloom, nor paralleling the bloom in significant concentration. Levels of odd-chain acids during the initiation of the waterbloom do not exceed levels noted in healthy Anabaena cultures with low background contamination. Kuentzel (1969) cites studies which suggest the importance of anticedent bacterial populations in their role as CO_2-providing symbionts in blue-green algal blooms. The fatty-acid findings would tend to ascribe a maintenance and "mopping-up" role to the bacteria, rather than an active role during initiation of the waterbloom. This is not meant to infer that the maintenance of a major bloom, lasting for weeks at a time, is an unimportant bacterial contribution. Additional subjective evidence of bacterial population increase after peak Anabaena concentrations comes from filtration of water samples. Following the peak there was a daily increase in the rate of filter-clogging although the planktonic elements were either stable or declining in numbers. As the glass-fiber filter (0.1 μ pore size) is an effective bacterial filter, the increased clogging was ascribed to increased bacterial concentration. Potaenko and Mikheeva (1969) noted inverse relationships between blue-green algal populations and bacteria, citing evidence from their own studies and those of other workers by Byelorussian lakes. His findings indicated, however, that increased eutrophication appeared to modify, and occasionally reverse, this relationship.

I exposed sterile conditioned medium and sterile extracted, conditioned medium (50 ml of medium in 125 ml Ehrlenmeyer flasks) to airborne contamination for several minutes, then refitted the cotton plugs and set the flasks aside. Within days, fungal and bacterial contamination began to overgrow the extracted medium. No appearance of contamination was noted in the conditioned medium after _four months_. This simple experiment has been repeated several times; the results are the same.

> The resulting high concentration of _A. flos-aquae_ at the surface, largely the result of extensive pseudovacuole formation, is recognized as a waterbloom; it is composed chiefly of senescent _Anabaena_ filaments.

In natural waterblooms, the color of _A. flos-aquae_ filaments ranges from brown to purple as a result of the light scattering properties of the gas filled pseudovacuoles. Upon centrifugation, the filaments displayed positive buoyancy, remaining at the top of the waters; a concentration of heterocysts was found at the bottom. The heterocysts did not appear to contain pseudovacuoles. Waaland and Waaland (1970) report light induction of pseudovacuole formation in _Nostoc muscorum_ - gas vacuoles were formed only when illumination exceeded 400 foot candles, and formation ceased when culture density lowered the mean illumination sufficiently. Light scattering properties of the gas vacuoles reduced light absorption according to the authors. While I did not observe pseudovacuole formation in laboratory _Anabaena_, the vacuole-inducing light value represents a level sufficient to induce degenerative changes.

Two additional factors may play a role in the rapid surface concentration of Anabaena. Hutchinson (1967) suggests a dying surge of multiplication under the supra-optimal conditions of light and temperature in surface waters. A second factor, noted consistently in the laboratory, is a positive phototactic response. If pseudovacuole formation in Anabaena should prove to be induced by high light conditions, then the observed phototaxis may indeed play a greater role in bringing Anabaena filaments to the surface than has heretofore been suspected.

Attempts to culture A. flos-aquae from surface waters met with consistent failure. As in old laboratory cultures, a senile degeneration appears to occur which is not reversible by transfer into fresh medium.

CONCLUSIONS

Anabaena flos-aquae can secrete a powerful broad-spectrum heteroinhibitor, which appears to prevent cell division in algae. A dramatic decline in phytoplankton populations accompanies the ascent of Anabaena to bloom concentrations; representative genera respond similarly in laboratory studies in medium conditioned by log-phase Anabaena. Growth of bacteria was suppressed in healthy cultures of Anabaena; data suggest such suppression exists in the natural system, also.

Lipid extracts of early bloom lake water containing fatty acids, readily assimilable by Anabaena, were stimulatory to the growth of the blue-green in laboratory studies. The stimulatory products are presumably released as other phytoplankton populations decomposed. Further studies are necessary to confirm the precise origin of the products in lake water extracts.

Phototaxis in Anabaena has been demonstrated and its role in surface concentration of a waterbloom has been suggested. As filaments from surface waters were incapable of further growth in all culture media tested, concentration by proliferation of filaments in surface waters is indeed doubtful. Buoyancy of pseudovacuole-filled Anabaena filaments was noted.

A simple bioassay procedure has been suggested for testing the nature and effect of Anabaena heteroinhibition. The next step must be an approach to extraction without inactivating the inhibitor. Bioassay with the active fractions can then aid in the purification and ultimate identification of these fractions.

Much remains unsolved. The precise conditions governing filament growth from akinetes, heterocyst production, phototactic behavior, and the formation of gas-filled pseudovacuoles are not known. However, a major role for heteroinhibition in the formation of Anabaena flos-aquae waterblooms is supported by my observations in the laboratory and in the natural system.

REFERENCES

Akehurst, S. C. 1931. Observations on Pond Life, With Special Preference to the Possible Causation of Swarming of Phytoplankton. J. Roy. Microscop. Soc. 51 (ser. 3):237-265.

Apstein, C. 1896. Das Susswasserplankton. Methods und Resultate der quantitativen Untersuchung. Kiel, vi + 200 pp.

Domogalla, B. P., C. Juday, and W. H. Patterson. 1925. The Forms of Nitrogen Found in Certain Lake Waters. J. Biol. Chem. 63:269-285.

Fitzgerald, George P. 1970. Evaluations of the Availability of Sources of Nitrogen and Phosphorus for Algae. J. Phycol. 6:239-247.

Fogg, G. E. 1949. Growth and Heterocyst Production in Anabaena cylindrica Lemm. II. In Relation to Carbon and Nitrogen Metabolism. Ann. Botany (London) 13:241-260.

Fogg, G. E. 1952. The Production of Extracellular Nitrogenous Substances by a Blue-green Alga. Roy. Soc. (London) Proc. B. 139:372-397.

Fogg, G. E. 1962. Extracellular Products. In: Physiology and Biochemistry of Algae (R. A. Lewin, ed.). Academic Press, New York. p. 475-489.

Gorham, Paul R. 1962. Laboratory Studies on the Toxins Produced by Waterblooms of Blue-green Algae. Amer. J. Publ. Health. 52(12): 2100-2105.

Hardy, A. C. 1936. Plankton Ecology and the Hypothesis of Animal Exclusion. Limnol. Soc. (London) Proc. 148:64-70.

Hartman, R. T. 1960. Algae and Metabolites of Natural Waters. The Pymatuning Symposia in Ecology: The Ecology of Algae. Spec. Publs. Pymatuning Lab. Fld. Biol. 2:38-55.

Hellebust, J. A. 1965. Excretion of Some Organic Compounds by Marine Phytoplankton. Limnol. Oceanogr. 10:192-206.

Hellebust, J. A. 1967. Excretion of Organic Compounds by Cultured and Natural Populations of Marine Phytoplankton. In: Estuaries (G. H. Lauff, ed.). Amer. Assoc. Advance. Sci. Publ. 83. Washington, D.C. p. 361-366.

Hoare, D. C., S. L. Hoare, and R. B. Moore. 1967. The Photoassimilation of Organic Compounds by Autotrophic Blue-green Algae. J. Gen. Microbiol. 49:351-370.

Hughes, E. O., P. R. Gorham, and A. Zehnder. 1958. Toxicity of a Unialgal Culture of Microcystis aeruginosa. Can. J. Microbiol. 4:225-236.

Hutchinson, G. E. 1957. A Treatise on Limnology, Vol. I. Geography, Physics, and Chemistry. John Wiley and Sons, New York. 1115 pp.

Hutchinson, G. E. 1967. A Treatise on Limnology, Vol. II. Introduction to Lake Biology and the Limnoplankton. John Wiley and Sons, New York. 1115 pp.

Jakob, H. 1961. Compatibilites, Antagonismes, et Antibioses Entre Quelques Algues du sol. Rev. Gen. Bot. 68:5-41.

Kuenzler, E. J. 1970. Dissolved Organic Phosphorus Excretion by Marine Phytoplankton. J. Phycol. 6(1):7-13.

Kuentzel, L. E. 1969. Bacteria, CO_2, and Algal Blooms. J. Water Poll. Con. Fed. 41:1737-1747.

Lange, W. 1967. Effect of Carbohydrates on the Symbiotic Growth of Planktonic Blue-green Algae with Bacteria. Nature. 215:1277-1278.

Lange, W. 1970. Cyanophyta-bacteria Systems: Effects of Added Carbon Compounds or Phosphate on Algal Growth at Low Nutrient Concentrations. J. Phycol. 6:230-234.

Lefevre, M., H. Jakob, and M. Nisbet. 1952. Autoet Heteroantagonisme Chez les Algues d'eau Douce. Ann. Stat. Centr. Hydrobiol. Appl. 4:5-197.

McNabb, C. D. 1960. Enumeration of Freshwater Phytoplankton Concentrated on the Membrane Filter. Limnol. Oceanogr. 5(1):57-61.

Meeuse, B. 1962. Storage Products. In: Physiology and Biochemistry of Algae (R. A. Lewin, ed.). Academic Press, New York.

Moore, B. G. and R. G. Tischer. 1965. Biosynthesis of Extracellular Polysaccharides by the Blue-green Alga Anabaena flos-aquae. Can. J. Microbiol. 11(6):877-885.

Moore, J. L., T. Richardson, and C. H. Amundson. 1964. Relative Response of Flame Ionization Detector to Short and Long Chain Fatty Acid Methyl Esters. J. Gas Chromatogr. 2:318-319.

Nichols, B. W., R. V. Harris, and A. T. James. 1965. The Lipid Metabolism of Blue-green Algae. Biochem. Biophys. Res. Commun. 20(3):256-262.

Ongun, A., W. W. Thomson, and J. B. Mudd. 1968. Lipid Composition of Chloroplasts Isolated by Aqueous and Non-aqueous Techniques. J. Lipid Res. 9:409.

Parker, P. L., C. Van Baalen, and L. Maurer. 1967. Fatty Acids in Eleven Species of Blue-green Algae: Geochemical Significance. Science. 155:707-708.

Pearsall, W. H. 1932. Phytoplankton in the English Lakes II. The Composition of the Phytoplankton in Relation to Dissolved Substances. J. Ecol. 20:241-262.

Potaenko, Yu. S., and T. M. Mikheeva. 1969. Relationships Between Bacteria and Phytoplankton. Microbiology. 38:603-607.

Proctor, V. W. 1957a. Some Controlling Factors in the Distribution of Haematococcus pluvialis. Ecology. 38:457-462.

Proctor, V. W. 1957b. Studies of Algal Antibiosis Using Haematococcus and Chlamydomonas. Limnol. Oceanogr. 2:125-139.

Vance, B. D. 1965. Composition and Succession of Cyanophycean Waterblooms. J. Phycol. 1:81-86.

Waaland, J. R., and S. D. Waaland. 1970. Light Induced Gas Vacuoles and Their Effect on Light Abosrption by the Blue-green Alga *Nostoc muscorum*. J. Phycol. 6[suppl.]:7.

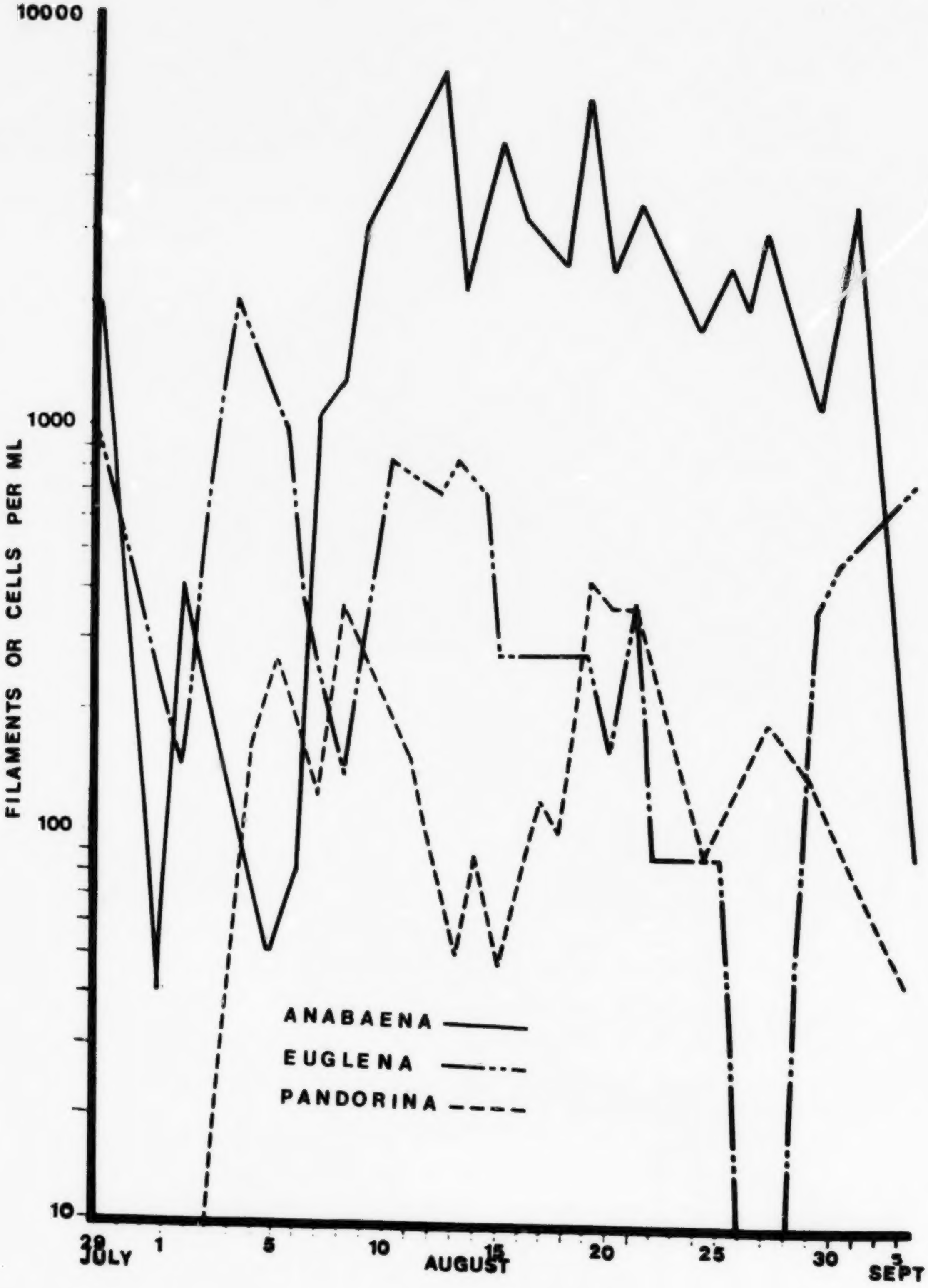
APPENDIX A - % Composition of Net Plankton
Lake Nelson - August 1970

Genera	8/8	8/10	8/11	8/12	8/15	8/17	8/18	8/19	8/20	8/21	8/24	8/25	8/26	8/27	8/28	8/29
Anabaena	8.4	88.6	91.0	84.3	68.5	48.0	37.6	57.8	50.1	25.1	64.6	57.9	21.8	10.9	6.6	3.2
Scenedesmus	3.9	tr	0.3	1.4	0.7	--	0.8	3.0	1.5	0.7	5.8	26.6	48.8	50.5	12.5	13.7
Euglena	--	tr	--	0.4	--	--	--	--	--	--	0.9	--	--	--	--	--
Pediastrum	2.3	tr	0.1	0.9	0.4	--	--	--	0.4	--	--	--	1.1	1.2	--	0.2
Dactylococcopsis	0.2	--	0.2	0.4	0.2	0.2	--	0.4	--	--	--	--	--	0.3	0.2	--
Pleodorina	0.7	2.1	2.5	2.7	1.3	0.2	--	--	--	1.1	0.5	--	--	--	3.7	4.4
Platydorina	0.1	tr	0.1	0.2	6.1	9.0	1.6	1.7	0.6	3.8	12.1	0.9	1.1	6.1	60.5	56.9
Eudorina	0.6	0.8	0.5	2.7	3.8	2.9	1.6	--	--	0.9	1.4	--	--	0.8	0.9	0.9
Micractinium	12.2	0.8	0.2	--	--	--	--	--	--	--	--	0.5	1.1	0.3	--	--
Golenkinia	1.1	tr	--	0.2	--	0.2	0.4	0.4	--	0.7	--	3.5	2.5	0.8	0.5	1.2
Chlorococcum	0.9	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Pandorina	--	--	0.2	0.2	0.4	0.5	0.4	0.1	0.4	--	--	0.5	--	--	--	--
Tetraedron	--	tr	0.1	0.4	0.2	0.5	--	0.4	--	--	--	--	0.4	0.3	0.2	0.2
Chlorogonium	--	tr	1.1	2.3	0.4	--	--	--	--	--	--	--	--	0.1	--	--
Phacus	0.1	tr	--	--	0.2	0.7	1.0	0.4	--	--	0.5	--	--	--	--	--

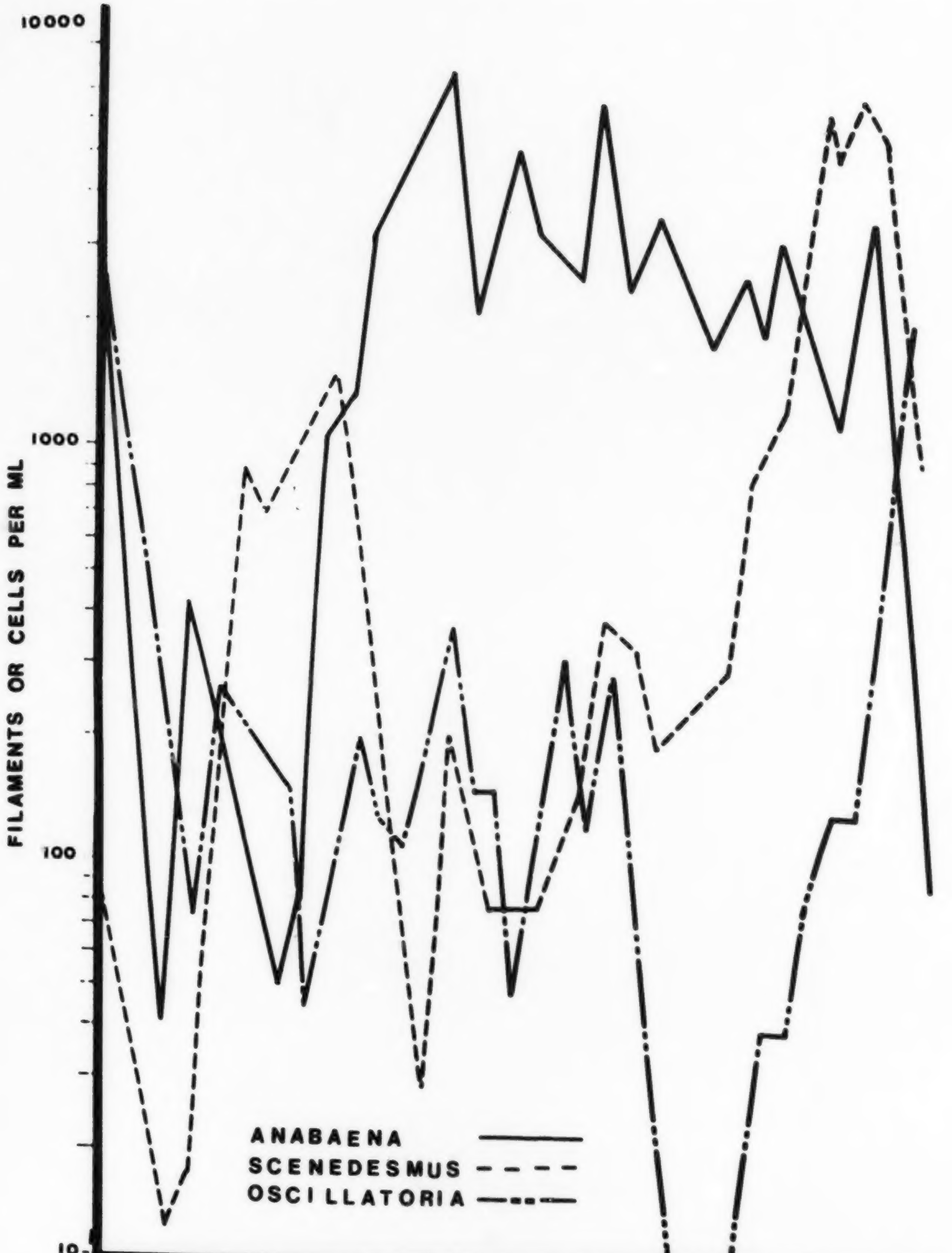
APPENDIX A (continued) - % Composition of Net Plankton
Lake Nelson - August 1970

Genera	8/8	8/10	8/11	8/12	8/15	8/17	8/18	8/19	8/20	8/21	8/24	8/25	8/26	8/27	8/28	8/29
Anacystis	0.1	--	--	--	0.4	1.1	16.2	9.2	10.1	13.0	4.5	0.9	1.4	1.5	2.3	3.5
Oscillatoria	0.9	1.3	2.6	4.5	2.7	1.8	2.0	5.7	2.2	2.5	1.8	0.9	1.8	1.8	1.1	3.2
Polyedriopsis	0.4	0.1	0.2	0.4	0.2	--	2.0	--	0.2	--	--	1.4	1.4	4.5	0.4	--
Actinastrum	0.7	0.1	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Filinia	1.8	0.1	--	--	8.1	22.2	17.7	7.5	26.0	30.3	0.9	--	2.2	1.1	1.6	2.0
Other Rotifers	52.3	1.7	1.0	1.4	3.4	10.9	20.3	13.0	7.5	20.9	7.2	1.9	5.0	0.7	0.5	8.0
Immature Copes	13.2	0.4	tr	0.5	2.7	1.9	0.4	--	--	0.4	--	--	0.4	--	--	0.2
Cladoceran	0.1	0.6	tr	--	--	--	--	0.1	--	--	--	--	--	--	--	--
Diatoms	--	1.6	--	--	--	--	--	0.3	--	--	--	0.5	1.8	--	--	--
Vorticella	--	--	--	--	--	--	--	--	--	0.7	--	2.3	0.7	1.5	--	--
Chlamydomonas	--	--	--	--	--	--	--	--	--	--	--	2.7	9.8	23.3	9.7	1.3

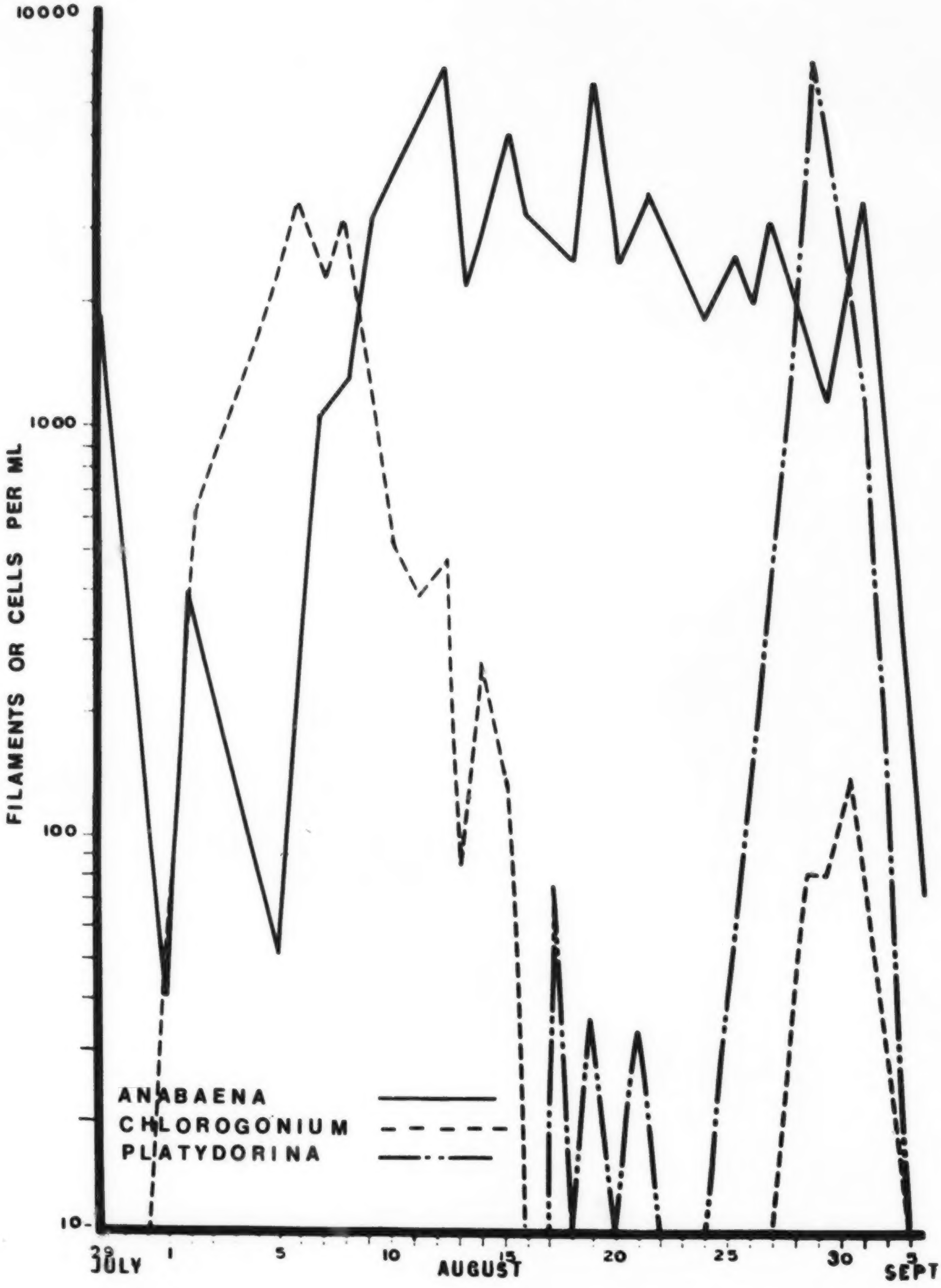
tr = 0.05% or less



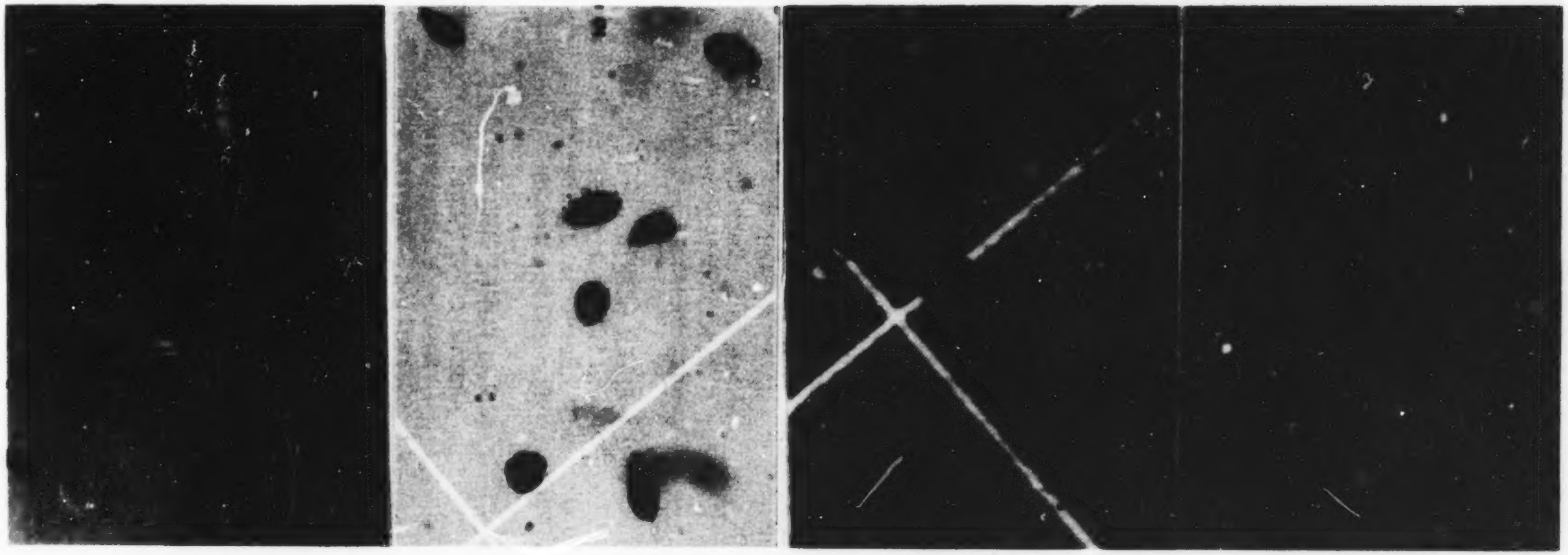
APPENDIX B - Variation of phytoplankton components during an Anabaena flos-aquae waterbloom in Lake Nelson, 1970.



APPENDIX C - Variation of phytoplankton components during an *Anabaena flos-aquae* waterbloom in Lake Nelson, 1970.



APPENDIX D - Variation of phytoplankton components during an *Anabaena flos-aquae* waterbloom in Lake Nelson, 1970.



APPENDIX E

Plate 1. *Euglena gracilis* grown in extracted conditioned medium. (100x)

Plate 2. *Euglena gracilis* grown in *Anabaena*-conditioned medium. (100x)

Plate 3. *Euglena gracilis* grown in extracted conditioned medium. (750x)

Plate 4. *Euglena gracilis* grown in *Anabaena*-conditioned medium. (750x)

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